

NOVEL FORMS OF T CELL COSTIMULATORY MOLECULES AND USES THEREFOR

Background of the Invention

5 For CD4+ T lymphocyte activation to occur, two distinct signals must be delivered by antigen presenting cells to resting T lymphocytes (Schwartz, R.H. (1990) *Science* 248:1349-1356; Williams, I.R. and Unanue, E.R. (1991) *J. Immunol.* 147:3752-3760; Mueller, D.L. et al., (1989) *J. Immunol.* 142:2617-2628). The first, or primary, activation signal is mediated physiologically by the interaction of the T cell receptor/CD3 complex
10 (TcR/CD3) with MHC class II-associated antigenic peptide and gives specificity to the immune response. The second signal, the costimulatory signal, regulates the T cell proliferative response and induction of effector functions. Costimulatory signals appear pivotal in determining the functional outcome of T cell activation since delivery of an antigen-specific signal to a T cell in the absence of a costimulatory signal results in functional
15 inactivation of mature T cells, leading to a state of tolerance (Schwartz, R.H. (1990) *Science* 248:1349-1356).

Molecules present on the surface of antigen presenting cells which are involved in T cell costimulation have been identified. These T cell costimulatory molecules include murine B7-1 (mB7-1; Freeman, G.J. et al., (1991) *J. Exp. Med.* 174:625-631), and the more recently
20 identified murine B7-2 (mB7-2; Freeman, G.J. et al., (1993) *J. Exp. Med.* 178:2185-2192). Human counterparts to the murine B7-1 and B7-2 molecules have also been described (human B7-1 (hB7-1) Freedman, A.S. et al., (1987) *J. Immunol.* 137:3260-3267; Freeman, G.J. et al., (1989) *J. Immunol.* 143:2714-2722; and human B7-2 (hB7-2); Freeman, G.J. et al., (1993) *Science* 262:909-911; Azuma, M. et al. (1993) *Nature* 366:76-79). The B7-1 and B7-
25 2 genes are members of the immunoglobulin gene superfamily.

B7-1 and B7-2 display a restricted pattern of cellular expression, which correlates with accessory cell potency in providing costimulation (Reiser, H. et al. (1992; *Proc. Natl. Acad. Sci. USA* 89:271-275; Razi-Wolf Z. et al., (1992) *Proc. Natl. Acad. Sci. USA* 89:4210-4214; Galvin, F. et al. (1992) *J. Immunol.* 149:3802-3808; Freeman, G.J. et al., (1993) *J. Exp. Med.* 178:2185-2192). For example, B7-1 has been observed to be expressed on activated B cells, T cells and monocytes but not on resting B cells, T cells or monocytes, and its
30 expression can be regulated by different extracellular stimuli (Linsley, P.S. et al., (1990) *Proc. Natl. Acad. Sci. USA* 87:5031-5035; Linsley, P.S. et al., (1991) *J. Exp. Med.* 174:561-569; Reiser, H. et al. (1992); *Proc. Natl. Acad. Sci. USA* 89:271-275; Gimmi, C.D. et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:6575-6579; Koulova, L. et al. (1991) *J. Exp. Med.* 173:759-762; Azuma, M. et al. (1993) *J. Exp. Med.* 177:845-850; Sansom, D.M. et al. (1993) *Eur. J. Immunol.* 23:295-298)

Both B7-1 and B7-2 are counter-receptors for two ligands, CD28 and CTLA4, expressed on T lymphocytes (Linsley, P.S. et al., (1990) *Proc. Natl. Acad. Sci. USA* 87:5031-

5035; Linsley, P.S. et al., (1991) *J. Exp. Med.* 174:561-569). CD28 is constitutively expressed on T cells and, after ligation by a costimulatory molecule, induces IL-2 secretion and T cell proliferation (June, C.H. et al. (1990) *Immunol. Today* 11:211-216). CTLA4 is homologous to CD28 and appears on T cells after activation (Freeman, G.J. et al. (1992) *J. Immunol.* 149:3795-3801). Although CTLA4 has a significantly higher affinity for B7-1 than does CD28, its role in T cell activation remains to be determined. It has been shown that antigen presentation to T cells in the absence of the B7-1/CD28 costimulatory signal results in T cell anergy (Gimmi, C.D. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6586-6590; Boussiotis, V.A. et al. (1993) *J. Exp. Med.* 178:1753). The ability of T cell costimulatory molecules such as B7-1 and B7-2 to bind to CD28 and/or CTLA4 on T cells and trigger a costimulatory signal in the T cells provides a functional role for these molecules in T cell activation.

Summary of the Invention

15 This invention pertains to novel forms of T cell costimulatory molecules. In particular, the invention pertains to isolated proteins encoded by T cell costimulatory molecule genes which contain amino acid sequences encoded by novel exons of these genes. The isolated proteins of the invention correspond to alternative forms of T cell costimulatory molecules. Preferably, these alternative forms correspond to naturally-occurring,
20 alternatively spliced forms of T cell costimulatory molecules or are variants of alternatively spliced forms which are produced by recombinant DNA techniques. The novel forms of T cell costimulatory molecules of the invention contain an alternative structural domain (i.e., a structural domain having an amino acid sequence which differs from a known amino acid sequence) or have a structural domain deleted or added. The occurrence in nature of
25 alternative structural forms of T cell costimulatory molecules supports additional functional roles for T cell costimulatory molecules.

The invention also provides isolated nucleic acid molecules encoding alternative forms of proteins which bind to CD28 and/or CTLA4 and isolated proteins encoded therein. Isolated nucleic acid molecules encoding polypeptides corresponding to novel structural
30 domains of T cell costimulatory molecules, and isolated polypeptide encoded therein are also within the scope of the invention. The novel structural domains of the invention are encoded by exons of T cell costimulatory molecule genes. In one embodiment of the invention, the T cell costimulatory molecule gene encodes B7-1. In another embodiment, the T cell costimulatory molecule gene encodes B7-2.

35 Another aspect of the invention provides proteins which bind CD28 and/or CTLA4 and contain a novel cytoplasmic domain. T cell costimulatory molecule genes which contain exons encoding different cytoplasmic domains which are used in an alternate manner have been discovered. Alternative splicing of mRNA transcripts of a T cell costimulatory molecule gene has been found to generate native T cell costimulatory molecules with

different cytoplasmic domains. The existence of alternative cytoplasmic domain forms of T cell costimulatory molecules supports a functional role for the cytoplasmic domain in transmitting an intracellular signal within a cell which expresses the costimulatory molecule on its surface. This indicates that costimulatory molecules not only trigger an intracellular signal in T cells, but may also deliver a signal to the cell which expresses the costimulatory molecule. This is the first evidence that the interaction between a costimulatory molecule on one cell and its receptor on a T cell may involve bidirectional signal transduction between the cells (rather than only unidirectional signal transduction to the T cell).

In yet another aspect of the invention, proteins that bind CD28 and/or CTLA4 and contain a novel signal peptide domain are provided. T cell costimulatory molecule genes which contain exons encoding different signal peptide domains which are used in an alternate manner have been discovered. Alternative splicing of mRNA transcripts of the gene can generate native T cell costimulatory molecules with different signal peptide domains. The existence of alternative signal peptide domain forms of T cell costimulatory molecules also suggests a functional role for the signal peptide of T cell costimulatory molecules.

Still another aspect of the invention pertains to isolated proteins that bind CD28 and/or CTLA4 in which a structural domain has been deleted or added, and isolated nucleic acids encoding such proteins. In a preferred embodiment, the protein (e.g., B7-1) has an immunoglobulin constant-like domain deleted (i.e., an immunoglobulin variable-like domain is linked directly to a transmembrane domain). In another embodiment, the protein has an immunoglobulin variable-like domain deleted (i.e., a signal peptide domain is linked directly to an immunoglobulin constant-like domain).

An isolated nucleic acid molecule of the invention can be incorporated into a recombinant expression vector and transfected into a host cell to express a novel structural form of a T cell costimulatory molecule. The isolated nucleic acids of the invention can further be used to create transgenic and homologous recombinant non-human animals. The novel T cell costimulatory molecules provided by the invention can be used to trigger a costimulatory signal in a T lymphocyte. These molecules can further be used to raise antibodies against novel structural domains of costimulatory molecules. The novel T cell costimulatory molecules of the invention can also be used to identify agents which stimulate the expression of alternative forms of costimulatory molecules and to identify components of the signal transduction pathway induced in a cell expressing a costimulatory molecule in response to an interaction between the costimulatory molecule and its receptor on a T lymphocyte.

Brief Description of the Drawings

Figure 1 is a photograph of an agarose gel depicting the presence of mB7-1 cytoplasmic domain II-encoding exon 6 in mB7-1 cDNA, determined by nested Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Figure 2 is a schematic representation depicting three mB7-1 transcripts (A, B and C) detected by nested RT-PCR.

Figure 3 is a graphic representation of interleukin-2 production by T cells stimulated with either untransfected CHO cells (CHO), CHO cells transfected to express full-length mouse B7-1 (CHO-B7-1) or CHO cells transfected to express the IgV-like isoform of mouse B7-1 (CHO-SV).

Detailed Description of the Invention

This invention pertains to novel structural forms of T cell costimulatory molecule which contain a structural domain encoded by a novel exon of a T cell costimulatory molecule gene, or have a structural domain deleted or added. Preferably, the isolated T cell costimulatory molecule corresponds to a naturally-occurring alternatively spliced form of a T cell costimulatory molecule, such as B7-1 or B7-2. Alternatively, the isolated protein can be a variant of a naturally-occurring alternatively spliced form of a T cell costimulatory molecule which is produced by standard recombinant DNA techniques.

Typically, a domain structure of a T cell costimulatory molecule of the invention includes a signal peptide domain (e.g., exon 1), an immunoglobulin variable region-like domain (IgV-like) (e.g., exon 2), an immunoglobulin constant region-like domain (IgC-like) (e.g. exon 3), a transmembrane domain (e.g., exon 4) and a cytoplasmic domain (e.g., exon 5). T cell costimulatory molecule genes are members of the immunoglobulin gene superfamily. The terms "immunoglobulin variable region-like domain" and "immunoglobulin constant region-like domain" are art-recognized and refer to protein domains which are homologous in sequence to an immunoglobulin variable region or an immunoglobulin constant region, respectively. For a discussion of the immunoglobulin gene superfamily and a description of IgV-like and IgC-like domains see Hunkapiller, T. and Hood, L. (1989) *Advances in Immunology* 44:1-63.

Each structural domain of a protein is usually encoded in genomic DNA by at least one exon. The invention is based, at least in part, on the discovery of novel exons in T cell costimulatory molecule genes which encode different forms of structural domains. Moreover, it has been discovered that exons encoding different forms of a structural domain of a T cell costimulatory molecule can be used in an alternative manner by alternative splicing of primary mRNA transcripts of a gene. Alternative splicing is an art-recognized term referring to the mechanism by which primary mRNA transcripts of a gene are processed to produce different mature mRNA transcripts encoding different proteins. In this mechanism different exonic sequences are excised from different primary transcripts. This results in mature mRNA transcripts from the same gene that contain different exonic sequences and thus encode proteins having different amino acid sequences. The terms "alternative forms" or "novel forms" of T cell costimulatory molecules refer to gene products of the same gene which differ in nucleotide or amino acid sequence from previously

disclosed forms of T cell costimulatory molecules, e.g., forms which result from alternative splicing of a primary mRNA transcript of a gene encoding a T cell costimulatory molecule.

Accordingly, one aspect of the invention relates to isolated nucleic acids encoding T cell costimulatory molecules corresponding to naturally-occurring alternatively spliced forms or variants thereof, and uses therefor. Another aspect of the invention pertains to novel structural forms of T cell costimulatory molecules which are produced by transcription and translation of the nucleic acid molecules of the invention, and uses therefor. This invention further pertains to isolated nucleic acids encoding novel structural domains of T cell costimulatory molecules, isolated polypeptides encoded therein, and uses therefor.

The various aspects of this invention are described in detail in the following subsections. Forming part of the present disclosure is the appended Sequence Listing. The numerous nucleotide and amino acid sequences presented in the Sequence Listing are summarized below.

- 15 SEQ ID NO: 1 - nucleotide sequence of mouse B7-1 exons 1-2-3-4-6
- SEQ ID NO: 2 - amino acid sequence of mouse B7-1 protein encoded by exons 1-2-3-4-6
- SEQ ID NO: 3 - nucleotide sequence of mouse B7-1 exons 1-2-3-4-5-6
- SEQ ID NO: 4 - nucleotide sequence of mouse B7-1 exon 6 (CytII)
- SEQ ID NO: 5 - amino acid sequence of mouse B7-1 peptide encoded by exon 6 (CytII)
- 20 SEQ ID NO: 6 - nucleotide sequence of mouse B7-1 full-length exon 1
- SEQ ID NO: 7 - nucleotide sequence of mouse B7-1 promoter
- SEQ ID NO: 8 - nucleotide sequence of B7-1 exons 1-3-4-5
- SEQ ID NO: 9 - amino acid sequence of mB7-1 protein encoded by exons 1-3-4-5
- SEQ ID NO: 10 - nucleotide sequence of mouse B7-1 exons 1-3-4-6
- 25 SEQ ID NO: 11 - amino acid sequence of mouse B7-1 protein encoded by exons 1-3-4-6
- SEQ ID NO: 12 - nucleotide sequence of mouse B7-2 exons m1B-2-3-4-5
- SEQ ID NO: 13 - amino acid sequence of mouse B7-2 protein encoded by exons m1B-2-3-4-5
- SEQ ID NO: 14 - nucleotide sequence of mouse B7-2 exon m1B
- SEQ ID NO: 15 - amino acid sequence of mouse B7-2 peptide encoded by exon m1B
- 30 SEQ ID NO: 16 - nucleotide sequence of mouse B7-1 exons 1-2-3-4-5 (as disclosed in Freeman, G. J. et al. (1991) *J. Exp. Med.* 174:625-631)
- SEQ ID NO: 17 - amino acid sequence of mouse B7-1 protein encoded by exons 1-2-3-4-5
- SEQ ID NO: 18 - nucleotide sequence of human B7-1 exons 1-2-3-4-5 (as disclosed in Freeman, G.J. et al. (1989) *J. Immunol.* 143:2714-2722)
- 35 SEQ ID NO: 19 - amino acid sequence of human B7-1 protein encoded by exons 1-2-3-4-5
- SEQ ID NO: 20 - nucleotide sequence of mouse B7-2 exons m1A-2-3-4-5 (as disclosed in Freeman, G.J. et al. (1993) *J. Exp. Med.* 178:2185-2192)
- SEQ ID NO: 21 - amino acid sequence of mouse B7-2 protein encoded by exons m1A-2-3-4-5

SEQ ID NO: 22 - nucleotide sequence of human B7-2 exons h1A-2-3-4-5 (as disclosed in Freeman, G.J. et al. (1993) *Science* 262:909-911)

SEQ ID NO: 23 - amino acid sequence of human B7-2 protein encoded by exons h1A-2-3-4-5

SEQ ID NO: 24 - nucleotide sequence of human B7-2 exons h1B-2-3-4-5 (as disclosed in

5 Azuma, M. et al. (1993) *Nature* 366:76-79)

SEQ ID NO: 25 - nucleotide sequence of mouse B7-1 exon 5 (Cyt I)

SEQ ID NO: 26 - amino acid sequence of mouse B7-1 peptide encoded by exon 5 (Cyt I)

SEQ ID NO: 27 - nucleotide sequence of human B7-1 exon 5 (Cyt I)

SEQ ID NO: 28 - amino acid sequence of human B7-1 peptide encoded by exon 5 (Cyt I)

10 SEQ ID NO: 29 - nucleotide sequence of mouse B7-2 exon 5 (Cyt I)

SEQ ID NO: 30 - amino acid sequence of mouse B7-2 peptide encoded by exon 5 (Cyt I)

SEQ ID NO: 31 - nucleotide sequence of human B7-2 exon 5 (Cyt I)

SEQ ID NO: 32 - amino acid sequence of human B7-2 peptide encoded by exon 5 (Cyt I)

SEQ ID NO: 33 - nucleotide sequence of mouse B7-1 truncated exon 1 (signal)

15 SEQ ID NO: 34 - amino acid sequence of mouse B7-1 peptide encoded by exon 1 (signal)

SEQ ID NO: 35 - nucleotide sequence of human B7-1 exon 1 (signal)

SEQ ID NO: 36 - amino acid sequence of human B7-1 peptide encoded by exon 1 (signal)

SEQ ID NO: 37 - nucleotide sequence of mouse B7-2 exon m1A (signal)

SEQ ID NO: 38 - amino acid sequence of mouse B7-2 peptide encoded by exon m1A (signal)

20 SEQ ID NO: 39 - nucleotide sequence of human B7-2 exon h1A (signal)

SEQ ID NO: 40 - amino acid sequence of human B7-2 peptide encoded by exon h1A (signal)

SEQ ID NO: 41 - nucleotide sequence of human B7-2 exon h1B (signal)

SEQ ID NO: 42 - amino acid sequence of human B7-2 peptide encoded by exon h1B (signal)

SEQ ID NOs: 43-61: oligonucleotide primers for PCR

25 SEQ ID NO: 62: nucleotide sequence of mouse B7-1 exons 1-2-4-5

SEQ ID NO: 63: nucleotide sequence of mouse B7-1 protein encoded by exons 1-2-4-5

SEQ ID NO: 64: nucleotide sequence of mouse B7-1 exons 1-2-4-6

SEQ ID NO: 65: nucleotide sequence of mouse B7-1 protein encoded by exons 1-2-4-6

30 I. Isolated Nucleic Acid Molecules Encoding T Cell Costimulatory Molecules

The invention provides an isolated nucleic acid molecule encoding a novel structural form of a T cell costimulatory molecule. As used herein, the term "T cell costimulatory molecule" is intended to include proteins which bind to CD28 and/or CTLA4. Preferred T cell costimulatory molecules are B7-1 and B7-2. The term "isolated" as used herein refers to

35 nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. An "isolated" nucleic acid is also free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the organism from which the nucleic acid is derived. The term "nucleic acid" is intended to include DNA

and RNA and can be either double stranded or single stranded. Preferably, the isolated nucleic acid molecule is a cDNA.

A. Nucleic Acids Encoding Novel Cytoplasmic Domains

One aspect of the invention pertains to isolated nucleic acids that encode T cell costimulatory molecules, each containing a novel cytoplasmic domain. It has been discovered that a gene encoding a costimulatory molecule can contain multiple exons encoding different cytoplasmic domains. In addition, naturally-occurring mRNA transcripts have been discovered which encode different cytoplasmic domain forms of T cell costimulatory molecules. Thus, one embodiment of the invention provides an isolated nucleic acid encoding a protein which binds CD28 or CTLA4 and comprises a contiguous nucleotide sequence derived from at least one T cell costimulatory molecule gene. In this embodiment, the nucleotide sequence can be represented by a formula A-B-C-D-E, wherein

A comprises a nucleotide sequence of at least one first exon encoding a signal peptide domain,

B comprises a nucleotide sequence of at least one second exon of a T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin variable region-like domain,

C comprises a nucleotide sequence of at least one third exon of a T cell costimulatory molecule gene, wherein the at least one third exon encodes an immunoglobulin constant region-like domain,

D comprises a nucleotide sequence of at least one fourth exon of a T cell costimulatory molecule gene, wherein the at least one fourth exon encodes a transmembrane domain, and

E comprises a nucleotide sequence of at least one fifth exon of a T cell costimulatory molecule gene, wherein the at least one fifth exon encodes a cytoplasmic domain,

with the proviso that E does not comprise a nucleotide sequence encoding a cytoplasmic domain selected from the group consisting of SEQ ID NO:25 (mB7-1), SEQ ID NO:27 (hB7-1), SEQ ID NO:29 (mB7-2) and SEQ ID NO:31 (hB7-2).

In the formula, A, B, C, D, and E are contiguous nucleotide sequences linked by phosphodiester bonds in a 5' to 3' orientation from A to E. According to the formula, A can be a nucleotide sequence of an exon which encodes a signal peptide domain of a heterologous protein which efficiently expresses transmembrane or secreted proteins, such as the oncostatin M signal peptide. Preferably, A comprises a nucleotide sequence of at least one exon which encodes a signal peptide domain of a T cell costimulatory molecule gene. It is

also preferred that A, B, C, D and E comprise nucleotide sequences of exons of the B7-1 gene, such as the human or murine B7-1 gene.

As described in detail in Examples 1 and 2, naturally-occurring murine B7-1 mRNA transcripts which contain a nucleotide sequence encoding one of at least two different cytoplasmic domains have been discovered. The alternative cytoplasmic domains are encoded in genomic DNA by different exons (i.e., either exon 5 or exon 6) and the different mB7-1 mRNA transcripts are produced by alternative splicing of the mRNA transcripts. The genomic structure of mB7-1 has been reported to contain only a single exon encoding cytoplasmic domain (i.e., exon 5; see Selvakumar, A. et al. (1993) *Immunogenetics* 38:292-295). The nucleotide sequence for the mB7-1 cDNA expressed in B cells has been reported to correspond to usage of five exons, 1-2-3-4-5 (the nucleotide sequence of which is shown in SEQ ID NO: 16) corresponding to signal, Ig-variable, Ig-constant, transmembrane and cytoplasmic domains (see Freeman, G.J. et al., (1991) *J. Exp. Med.* 174:625-631). This transcript includes a single exon encoding cytoplasmic domain, exon 5. As described herein, the nucleotide sequence of a sixth exon for the mB7-1 gene which encodes a cytoplasmic domain having a different amino acid sequence than the cytoplasmic domain encoded by exon 5 has been discovered. The nucleotide sequence encoding the first cytoplasmic domain of mB7-1 (i.e., exon 5) is shown in SEQ ID NO: 25 and the amino acid sequence of this cytoplasmic domain (referred to herein as Cyt I) is shown in SEQ ID NO: 26. A nucleotide sequence encoding a second, alternative cytoplasmic domain for mB7-1 (i.e., exon 6) is shown in SEQ ID NO: 4. This alternative cytoplasmic domain encoded by exon 6 (also referred to herein as Cyt II) has an amino acid sequence shown in SEQ ID NO: 5.

The Cyt II domain of mB7-1 has several characteristic properties. Of interest is the preferential expression of mRNA containing the exon encoding Cyt II (i.e., exon 6) in thymus. In contrast, mRNA containing exon 6 of mB7-1 is not detectable in spleen. Accordingly, this invention encompasses alternative cytoplasmic domain forms of T cell costimulatory molecules which are expressed preferentially in thymus. As defined herein, the term "expressed preferentially in the thymus" is intended to mean that the mRNA is detectable by standard methods in greater abundance in the thymus than in other tissues which express the T cell costimulatory molecule, particularly the spleen. The Cyt II domain of mB7-1 has also been found to contain several consensus phosphorylation sites and, thus, alternative cytoplasmic domain forms of T cell costimulatory molecules which contain at least one consensus phosphorylation site are also within the scope of this invention. As used herein, the term "consensus phosphorylation site" describes an amino acid sequence motif which is recognized by and phosphorylated by a protein kinase, for example protein kinase C, casein kinase II etc. It has also been discovered that exon 6 is encoded in genomic DNA approximately 7.5 kilobases downstream of exon 5. This invention therefore includes alternative cytoplasmic domain forms of T cell costimulatory molecules which are located in genomic DNA less than approximately 10 kb downstream (i.e., 3') of an exon encoding a first

cytoplasmic domain of the T cell costimulatory molecule. Additionally, a second, alternative cytoplasmic domain of another T cell costimulatory molecule is likely to be homologous to the Cyt II domain of mB7-1. For example, the first cytoplasmic domains of mB7-1, hB7-1, mB7-2 and hB7-2 display between 4 % and 26 % amino acid identity (see Freeman, G.J. et al. (1993) *J. Exp. Med.* 178:2185-2192). Accordingly, in one embodiment, an alternative cytoplasmic domain of a T cell costimulatory molecule has an amino acid sequence that is at least about 5 % to 25 % identical in sequence with the amino acid sequence of mB7-1 Cyt II (shown in SEQ ID NO: 5).

Another embodiment of the invention provides an isolated nucleic acid encoding a protein which binds CD28 or CTLA4 and is encoded by a T cell costimulatory molecule gene having at least one first exon encoding a first cytoplasmic domain and at least one second exon encoding a second cytoplasmic domain. The at least one first cytoplasmic domain exon of the gene comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence of SEQ ID NO:25 (mB7-1), SEQ ID NO:27 (hB7-1), SEQ ID NO:29 (mB7-2) and SEQ ID NO:31 (hB7-2). In this embodiment, the isolated nucleic acid includes a nucleotide sequence encoding at least one second cytoplasmic domain. Preferably, the isolated nucleic acid does not comprise a nucleotide sequence encoding a first cytoplasmic domain (i.e., the nucleic acid comprises an alternative splice form of a transcript of the gene in which the exon encoding the first cytoplasmic domain, e.g., exon 5, has been excised from the transcript). Preferred T cell costimulatory molecule genes from which nucleotide sequences can be derived include B7-1 and B7-2.

In yet another embodiment, the isolated nucleic acid of the invention encodes a protein which binds CD28 or CTLA4 and comprises a nucleotide sequence shown in SEQ ID NO: 1. This nucleotide sequence corresponds to a naturally-occurring alternatively spliced form of mB7-1 which includes the nucleotide sequences of exons 1-2-3-4-6. Alternatively, the isolated nucleic acid comprises a nucleotide sequence shown in SEQ ID NO: 3, which corresponds to a naturally-occurring alternatively spliced form of mB7-1 comprising the nucleotide sequences of exons 1-2-3-4-5-6.

B. Nucleic Acids Encoding Novel Signal Peptide Domains

Other aspects of this invention pertain to isolated nucleic acids which encode T cell costimulatory molecules containing novel signal peptide domains. It has been discovered that a gene encoding a costimulatory molecule can contain multiple exons encoding different signal peptide domains and that mRNA transcripts occur in nature which encode different signal peptide domain forms of T cell costimulatory molecules. Thus, isolated nucleic acids which encode proteins which bind CD28 or CTLA4 and comprise contiguous nucleotide sequences derived from at least one T cell costimulatory molecule gene are within the scope of this invention. The nucleotide sequence can be represented by a formula A-B-C-D-E, wherein

A comprises a nucleotide sequence of at least one first exon of a T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

5 B comprises a nucleotide sequence of at least one second exon of a T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin variable region-like domain,

C comprises a nucleotide sequence of at least one third exon of a T cell costimulatory molecule gene, wherein the at least one third exon encodes an
10 immunoglobulin constant region-like domain,

D, which may or may not be present, comprises a nucleotide sequence of at least one fourth exon of a T cell costimulatory molecule gene, wherein the at least one fourth exon encodes a transmembrane domain, and

E, which may or may not be present, comprises a nucleotide sequence of at
15 least one fifth exon of a T cell costimulatory molecule gene, wherein the at least one fifth exon encodes a cytoplasmic domain,

with the proviso that A does not comprise a nucleotide sequence encoding a signal peptide domain selected from the group consisting of SEQ ID NO:33 (mB7-1), SEQ ID NO:35 (hB7-
20 1), SEQ ID NO:37 (mB7-2), SEQ ID NO:39 (hB7-2) and SEQ ID NO:41 (hB7-2).

In the formula, A, B, C, D, and E are contiguous nucleotide sequences linked by phosphodiester bonds in a 5' to 3' orientation from A to E. To produce a soluble form of the T cell costimulatory molecule D, which comprises nucleotide sequence of a transmembrane domain and E, which comprises a nucleotide sequence of a cytoplasmic domain may not be
25 present in the molecule. In a preferred embodiment, A, B, C, D and E comprise nucleotide sequences of exons of the B7-2 gene, such as the human or murine B7-2 gene.

As described in detail in Example 6, naturally-occurring murine B7-2 mRNA transcripts which contain a nucleotide sequence encoding one of at least two different signal peptide domains have been discovered. One of these signal domains corresponds to the
30 signal domain of murine B7-2 disclosed in Freeman et al. (1993) *J. Exp. Med.* 178:2185-2192 (this signal domain is referred to herein as exon m1A). However, the second signal domain corresponds to a novel nucleotide sequence (referred to herein as m1B). Accordingly, an mRNA transcript containing a nucleotide sequence encoding the novel signal peptide domain (m1B) represents an alternatively spliced form of murine B7-2. A naturally-occurring mB7-2
35 mRNA transcript comprising the alternative signal peptide domain (i.e., comprising exons m1B-2-3-4-5) preferably comprises the nucleotide sequence shown in SEQ ID NO: 12, and encodes a protein comprising the amino acid sequence shown in SEQ ID NO: 13. The nucleotide and amino acid sequences of the novel signal peptide domain (i.e., exon m1B) are shown in SEQ ID NOs: 14 and 15, respectively.

In yet another embodiment of the invention, the isolated nucleic acid encodes a protein which binds CD28 or CTLA4 and is encoded by a T cell costimulatory molecule gene having at least one first exon encoding a first signal peptide domain and at least one second exon encoding a second signal peptide domain. The at least one first exon comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence of SEQ ID NO:33 (mB7-1), SEQ ID NO:35 (hB7-1), SEQ ID NO:37 (mB7-2) and SEQ ID NO:39 (hB7-2) and SEQ ID NO:41 (hB7-2). In this embodiment, the isolated nucleic acid includes a nucleotide sequence encoding at least one second signal peptide domain. Preferably, the isolated nucleic acid does not comprise a nucleotide sequence encoding the first signal peptide domain (i.e., the nucleic acid comprises an alternative splice form of a transcript of the gene in which the exon encoding a first signal domain has been excised from the transcript). Preferred T cell costimulatory molecule gene from which nucleotide sequences can be derived include B7-1 and B7-2.

C. Nucleic Acids Encoding Proteins With Domains Deleted or Added

Another aspect of the invention pertains to isolated nucleic acids encoding T cell costimulatory molecules having structural domains which have been deleted or added. This aspect of the invention is based, at least in part, on the discovery that alternative splicing of mRNA transcripts encoding T cell costimulatory molecules generates transcripts in which an exon encoding a structural domain has been excised or in which at least two exons encoding two forms of a structural domain are linked in tandem. In one embodiment, the nucleic acid is one in which an exon encoding an IgV-like domain has been deleted (i.e., the signal peptide domain exon is linked directly to the IgC-like domain exon). Accordingly, in one embodiment, the isolated nucleic acid encodes a protein comprising a contiguous nucleotide sequence derived from at least one T cell costimulatory molecule gene, the nucleotide sequence represented by a formula A-B-C-D, wherein

A comprises a nucleotide sequence of at least one first exon of a T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

B comprises a nucleotide sequence of at least one second exon of a T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin constant region-like domain,

C comprises a nucleotide sequence of at least one third exon of a T cell costimulatory molecule gene, wherein the at least one third exon encodes a transmembrane domain, and

D comprises a nucleotide sequence of at least one fourth exon of a T cell costimulatory molecule gene, wherein the at least one fourth exon encodes a cytoplasmic domain.

In the formula, A, B, C and D are contiguous nucleotide sequences linked by phosphodiester bonds in a 5' to 3' orientation from A to D.

Naturally-occurring mRNA transcripts encoding murine B7-1 have been detected in which the exon encoding the IgV-like domain (i.e., exon 2) has been excised and the exon encoding the signal peptide domain (i.e., exon 1) is spliced to the exon encoding the IgC-like domain (i.e., exon 3) (see Example 7). In one embodiment, an isolated nucleic acid encoding an alternatively spliced form of murine B7-1 in which an IgV-like domain exon has been deleted comprises a nucleotide sequence corresponding to usage of exons 1-3-4-5 (SEQ ID NO: 8). Alternatively, an alternatively spliced form of murine B7-1 comprises a nucleotide sequence corresponding to usage of exons 1-3-4-6 (SEQ ID NO: 10), which contains the second, alternative cytoplasmic domain of mB7-1.

In another embodiment, nucleic acid is one in which an exon encoding an IgC-like domain has been deleted (i.e., the IgV-like domain exon is linked directly to the transmembrane domain exon). Accordingly, in one embodiment, the isolated nucleic acid encodes a protein comprising a contiguous nucleotide sequence derived from at least one T cell costimulatory molecule gene, the nucleotide sequence represented by a formula A-B-C-D, wherein

A comprises a nucleotide sequence of at least one first exon of a T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

B comprises a nucleotide sequence of at least one second exon of a T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin variable region-like domain,

C comprises a nucleotide sequence of at least one third exon of a T cell costimulatory molecule gene, wherein the at least one third exon encodes a transmembrane domain, and

D comprises a nucleotide sequence of at least one fourth exon of a T cell costimulatory molecule gene, wherein the at least one fourth exon encodes a cytoplasmic domain.

In the formula, A, B, C and D are contiguous nucleotide sequences linked by phosphodiester bonds in a 5' to 3' orientation from A to D.

In one embodiment, an isolated nucleic acid encoding an alternatively spliced form of murine B7-1 in which an IgC-like domain exon has been deleted comprises a nucleotide sequence corresponding to usage of exons 1-2-4-5 (shown in SEQ ID NO: 62). The amino acid sequence of the protein encoded by this nucleic acid is shown in SEQ ID NO: 63. Moreover, in another embodiment, an alternatively spliced form of murine B7-1 in which an IgC-like domain exon has been deleted can comprise a nucleotide sequence corresponding to usage of exons 1-2-4-6 (shown in SEQ ID NO: 64), which contains the second, alternative

cytoplasmic domain of mB7-1. The amino acid sequence of the protein encoded by this nucleic acid is shown in SEQ ID NO: 65. Naturally-occurring mRNA transcripts encoding murine B7-1 have been detected in which the exon encoding the IgC-like domain (i.e., exon 3) has been excised and the exon encoding the IgV-like domain (i.e., exon 2) is spliced to the exon encoding the transmembrane domain (i.e., exon 4) (see Example 7). When expressed in a host cell, the IgV-like isoform of mB7-1 is capable of binding to both mouse CTLA4 and mouse CD28 and can trigger a costimulatory signal in a T cell such that the T cell proliferates and produces interleukin-2 (see Example 7).

Yet another aspect of this invention features an isolated nucleic acid encoding a T cell costimulatory molecule which contains exons in addition to a known or previously identified form of the T cell costimulatory molecule. For example, a naturally-occurring murine B7-1 mRNA transcript has been identified which contains two cytoplasmic domain-encoding exons in tandem, i.e., the transcript contains exons 1-2-3-4-5-6 (the nucleotide sequence of which is shown in SEQ ID NO: 3). Since there is an in-frame termination codon within exon 5, translation of this transcript produces a protein which contains only the Cyt I cytoplasmic domain. However, if desired, this termination codon can be mutated by standard site-directed mutagenesis techniques to create a nucleotide sequence which encodes an mB7-1 protein containing both a Cyt I and a Cyt II domain in tandem.

II. Isolation of Nucleic Acids of the Invention

An isolated nucleic acid having a nucleotide sequence disclosed herein can be obtained by standard molecular biology techniques. For example, oligonucleotide primers suitable for use in the polymerase chain reaction (PCR) can be prepared based upon the nucleotide sequences disclosed herein and the nucleic acid molecule can be amplified from cDNA and isolated. At least one oligonucleotide primer should be complimentary to a nucleotide sequence encoding an alternative structural domain. It is even more preferable that at least one oligonucleotide primer span a novel exon junction created by alternative splicing. For example, an oligonucleotide primer which spans the junction of exon 4 and exon 6 can be used to preferentially amplify a murine B7-1 cDNA that contains the second, alternative cytoplasmic domain (e.g., a cDNA which contains exons 1-2-3-4-6; SEQ ID NO: 1). Alternatively, an oligonucleotide primer complimentary to a nucleotide sequence encoding a novel alternative structural domain can be used to screen a cDNA library to isolate a nucleic acid of the invention.

Isolated nucleic acid molecules having nucleotide sequences other than those specifically disclosed herein are also encompassed by the invention. For example, novel structural forms of B7-1 from species other than mouse are within the scope of the invention (e.g., alternatively spliced forms of human B7-1). Likewise, novel structural forms of B7-2 from species other than mouse are also within the scope of the invention (e.g., alternatively spliced forms of human B7-2). Furthermore, additional alternatively spliced forms for

murine B7-1 and murine B7-2 can be identified using techniques described herein. These alternatively spliced forms of murine B7-1 and B7-2 are within the scope of the invention. Isolated nucleic acid molecules encoding novel structural forms of T cell costimulatory molecules can be obtained by conventional techniques, such as by methods described below and in the Examples.

An isolated nucleic acid encoding a novel structural form of a T cell costimulatory molecule can be obtained by isolating and analyzing cDNA clones encoding the T cell costimulatory molecule (e.g., mB7-1; hB7-1; mB7-2; hB7-2 etc.) by standard techniques (see for example Sambrook *et al.* *Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory press (1989) or other laboratory handbook). For example, cDNAs encoding the costimulatory molecule can be amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using oligonucleotide primers specific for the costimulatory molecule gene. The amplified cDNAs can then be subcloned into a plasmid vector and sequenced by standard methods. Oligonucleotide primers for RT-PCR can be designed based upon previously disclosed nucleotide sequences of costimulatory molecules (see Freeman, G.J. et al., (1991) *J. Exp. Med.* 174:625-631 for mB7-1; Freeman, G.J. et al., (1989) *J. Immunol.* 143:2714-2722 for hB7-1; Freeman, G.J. et al., (1993) *J. Exp. Med.* 178:2185-2192 for mB7-2; and Freeman, G.J. et al., (1993) *Science* 262:909-911 for hB7-2; nucleotide sequences are shown in SEQ ID NOS: 16, 18, 20, 22 and 24). For analyzing the 5' or 3' ends of mRNA transcripts, cDNA can be prepared using a 5' or 3' "RACE" procedure ("rapid amplification of cDNA ends) as described in the Examples. Alternative to amplifying specific cDNAs, a cDNA library can be prepared from a cell line which expresses the costimulatory molecule and screened with a probe containing all or a portion of the nucleotide sequence encoding the costimulatory molecule.

Individual isolated cDNA clones encoding a T cell costimulatory molecule can then be sequenced by standard techniques, such as dideoxy sequencing or Maxam-Gilbert sequencing, to identify a cDNA clone encoding a T cell costimulatory molecule having a novel structural domain. A novel structural domain can be identified by comparing the sequence of the cDNA clone to the previously disclosed nucleotide sequences encoding T cell costimulatory molecules (e.g., sequences shown in SEQ ID NO: 16, 18, 20, 22 and 24). Once a putative alternative structural domain has been identified, the nucleotide sequence encoding the domain can be mapped in genomic DNA to determine whether the domain is encoded by a novel exon. This type of approach provides the most extensive information about alternatively spliced forms of mRNAs encoding the costimulatory molecule.

Alternatively, a novel structural domain for T cell costimulatory molecules can be identified in genomic DNA by identifying a novel exon in the gene encoding the T cell costimulatory molecule. A novel exon can be identified as an open reading frame flanked by splice acceptor and splice donor sequences. Genomic clones encoding a T cell costimulatory molecule can be isolated by screening a genomic DNA library with a probe encompassing all

or a portion of a nucleotide sequence encoding the costimulatory molecule (e.g., having all or a portion of a nucleotide sequence shown in SEQ ID NO: 16, 18, 20, 22 and 24). For costimulatory molecules whose genes have been mapped to a particular chromosome, a chromosome-specific library rather than a total genomic DNA library can be used. For example, hB7-1 has been mapped to human chromosome 3 (see Freeman, G.J. et al. (1992) *Blood* 79:489-494; and Selvakumar, A. et al. (1992) *Immunogenetics* 36:175-181. Genomic clones can be sequenced by conventional techniques and novel exons identified. A probe corresponding to a novel exon can then be used to detect the nucleotide sequence of this exon in mRNA transcripts encoding the costimulatory molecule (e.g., by screening a cDNA library or by PCR).

A more preferred approach for identifying and isolating nucleic acid encoding a novel structural domain of a T cell costimulatory molecule is by "exon trapping". Exon trapping is a technique that has been used successfully to identify and isolate novel exons (see e.g. Duyk, G.M. et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:8995-8999; Auch, D. and Reth, M. (1990) *Nucleic Acids Res.* 18:6743-6744; Hamaguchi, M. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:9779-9783; and Krizman, D.B and Berget, S.M. (1993) *Nucleic Acids Res.* 21:5198-5202). The approach of exon trapping can be applied to the isolation of exons encoding novel structural domains of T cell costimulatory molecules, such as a novel alternative cytoplasmic domain of human B7-1, as described in Example 5.

In addition to the isolated nucleic acids encoding naturally-occurring alternatively spliced forms of T cell costimulatory molecules provided by the invention, it will be appreciated by those skilled in the art that nucleic acids encoding variant alternative forms, which may or may not occur naturally, can be obtained using standard recombinant DNA techniques. The term "variant alternative forms" is intended to include novel combinations of exon sequences which can be created using recombinant DNA techniques. That is, novel exons encoding structural domains of T cell costimulatory molecules, either provided by the invention or identified according to the teachings of the invention, can be "spliced", using standard recombinant DNA techniques, to other exons encoding other structural domains of the costimulatory molecule, regardless of whether the particular combination of exons has been observed in nature. Thus, novel combinations of exons can be linked *in vitro* to create variant alternative forms of T cell costimulatory molecules. For example, the structural form of murine B7-1 which has the signal peptide domain directly joined to the IgC-like domain (ie., which has the IgV-like domain deleted) has been observed in nature in combination with the cytoplasmic domain encoded by exon 5. However, using conventional techniques, an alternative structural form can be created in which the IgV-like domain is deleted and the alternative cytoplasmic domain is encoded by exon 6. In another example, a murine B7-1 cDNA containing exons 1-2-3-4-5-6 can be mutated by site-directed mutagenesis to change a stop codon in exon 5 to an amino acid encoding-codon such that an mB7-1 protein can be produced which contains both a Cyt I domain and a Cyt II domain in tandem. Additionally,

an exon encoding a structural domain of one costimulatory molecule can be transferred to another costimulatory molecule by standard techniques. For example, the cytoplasmic domain of mB7-2 can be replaced with the novel cytoplasmic domain of mB7-1 provided by the invention (i.e., exon 6 of mB7-1 can be "swapped" for the cytoplasmic domain exon of mB7-2).

For the purposes of this invention, the amino acid residues encompassing the different "domains" or "exons" (i.e., signal (S), IgV-like (V), IgC-like (C), transmembrane (TM) and cytoplasmic (Cyt)) of mouse and human B7-1 and B7-2 proteins are defined as follows: mouse B7-1 (as shown in SEQ ID NO: 17): ~1-37 (S), ~38-142 (V), ~143-247 (C), ~248-274 (TM) and ~275-306 (Cyt); human B7-1 (as shown in SEQ ID NO: 19): ~1-33 (S), ~34-138 (V), ~139-242 (C), ~243-265 (TM) and ~266-288 (Cyt); mouse B7-2 (as shown in SEQ ID NO: 21): ~1-5 (S), ~6-133 (V), ~134-233 (C), ~234-264 (TM) and ~265-309 (Cyt); and human B7-2 (as shown in SEQ ID NO: 23): ~1-6-22 (S), ~23-132 (V), ~133-245 (C), ~246-268 (TM) and ~269-329 (Cyt). It will be appreciated by the skilled artisan that regions slightly longer or shorter than these amino acid domains (i.e., a few amino acid residues more or less at either the amino-terminal or carboxy-terminal end) may be equally suitable for use as signal, IgV-like, IgC-like, transmembrane and/or cytoplasmic domains in the proteins of the invention (i.e., there is some flexibility in the junctions between different domains within the proteins of the invention as compared to the domain junctions delineated above for B7-1 and B7-2 proteins). Accordingly, proteins comprising signal, IgV-like, IgC-like, transmembrane and/or cytoplasmic domains having essentially the same amino acid sequences as those regions delineated above but which differ from the above-delineated junctions merely be a few amino acid residues, either longer or shorter, at either the amino- or carboxy-terminal end of the domain are intended to be encompassed by the invention. Nucleic acid segments encoding any of the domains delineated above can be obtained by standard techniques, e.g., by PCR amplification using oligonucleotide primers based on the nucleotide sequences disclosed herein, and can be ligated together to create nucleic acid molecules encoding recombinant forms of the proteins of the invention.

It will also be appreciated by those skilled in the art that changes can be made in the nucleotide sequences provided by the invention without changing the encoded protein due to the degeneracy of the genetic code. Additionally, nucleic acids which have a nucleotide sequence different from those disclosed herein due to degeneracy of the genetic code may be isolated from biological sources. Such nucleic acids encode functionally equivalent proteins (e.g., a protein having T cell costimulatory activity) to those described herein. For example, a number of amino acids are designated by more than one triplet codon. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may occur in isolated nucleic acids from different biological sources or can be introduced into an isolated nucleic acid by standard recombinant DNA techniques without changing the protein encoded by the nucleic acid. Isolated nucleic acids encoding alternatively spliced

forms of T cell costimulatory molecules having a nucleotide sequence which differs from those provided herein due to degeneracy of the genetic code are considered to be within the scope of the invention.

5 III. Additional Isolated Nucleic Acid Molecules of the Invention

In addition to isolated nucleic acids encoding alternative forms of T cell costimulatory molecules, the invention also discloses previously undescribed nucleotide sequences of the murine B7-1 gene and mRNA transcripts. As described in detail in Example 3, it has now been discovered that murine B7-1 mRNA transcripts contain additional 5' untranslated (UT) sequences which were not previously reported. A 5' UT region of approximately 250 base pairs has been reported for mB7-1 mRNA transcripts, determined by primer extension analysis (see Selvakumar et al. (1993) *Immunogenetics* 38:292-295). As described herein, an additional ~1500 nucleotides of 5' UT sequences have been discovered in mB7-1. These 5' UT sequences are contiguous with known exon 1 sequences, thereby extending the size of exon 1 by approximately 1500 base pairs. Thus the novel 5' UT sequence of the invention corresponds to the 5' region of mB7-1 exon 1 (i.e., exon 1 extends an additional ~1500 nucleotides at its 5' end than previously reported) rather than corresponding to a new exon upstream of exon 1. Computer analysis of the potential secondary structure of the 5' UT region reveals that the most stable structure is comprised of multiply folded palindromic sequences. This high degree of secondary structure may explain the results of Selvakumar et al. ((1993) *Immunogenetics* 38:292-295) in that the secondary structure could account for premature termination of the primer extension reaction. The potential for excessive secondary structure in the 5' UT region suggests that post-transcriptional mechanisms are involved in controlling mB7-1 expression. Thus, inclusion of the long 5' UT sequence in recombinant expression vectors encoding mB7-1 may provide post-transcriptional regulation that is similar to that of the endogenous gene. Accordingly, the 5' UT region of mB7-1 provided by the invention can be incorporated by standard recombinant DNA techniques at the 5' end of a cDNA encoding a mB7-1 protein. The nucleotide sequence of the 5' UT region of mB7-1 (i.e., the full nucleotide sequence of exon 1) is shown in SEQ ID NO: 6.

The discovery of additional 5' UT sequences in mB7-1 cDNA demonstrates that transcription of the mB7-1 gene initiates further upstream (i.e., 5') in genomic DNA than previously reported in Selvakumar et al. (*Immunogenetics* (1993) 38:292-295). Transcription of a gene is typically regulated by sequences in genomic DNA located immediately upstream of sequences corresponding to the 5' UT region of the transcribed mRNA. Nucleotides located within approximately 200 base pairs of the start site of transcription are generally considered to encompass the promoter of the gene and often include canonical CCAAT or TATA elements indicative of a typical eukaryotic promoter. For a gene having a promoter which contains a TATA box, transcription usually starts approximately 30 base pairs downstream of the TATA box. In addition to CCAAT and TATA-containing promoters, it is

now appreciated that many genes have promoters which do not contain these elements. Examples of such genes include many members of the immunoglobulin gene superfamily (see for example Breathnach, R. and Chambon, P. (1981) *Ann. Rev. Biochem.* 50:349-383; Fisher, R.C. and Thorley-Lawson, D.A. (1991) *Mol. Cell. Biol.* 11:1614-1623; Hogarth, P.M. et al. (1991) *J. Immunol.* 146:369-376; Schanberg, L.E. (1991) *Proc. Natl. Acad. Sci. USA* 88:603-607; Zhou, L.J. et al. (1991) *J. Immunol.* 147:1424-1432). In such TATA-less promoters, transcriptional regulation is thought to be provided by other DNA elements which bind transcription factors. Sequence analysis of ~180 base pairs of mB7-1 genomic DNA immediately upstream of the newly identified 5' UT region revealed the presence of numerous consensus sites for transcription factor binding, including AP-2, PU.1 and NFκB. The nucleotide sequence of this region is shown in SEQ ID NO: 7. The structure of this region (i.e, the DNA elements contained therein) is consistent with it functioning as a promoter for transcription of the mB7-1 gene. The ability of this region of DNA to function as a promoter can be determined by standard techniques routinely used in the art to identify transcriptional regulatory elements. For example, this DNA region can be cloned upstream of a reporter gene (e.g., encoding chloramphenicol acetyl transferase, β-galactosidase, luciferase etc.) in a recombinant vector, the recombinant vector transfected into an appropriate cell line and expression of the reporter gene detected as an indication that the DNA region can function as a transcriptional regulatory element. If it is determined that this DNA region can function as a B7-1 promoter, it may be advantageous to use this DNA region to regulate expression of a B7-1 cDNA in a recombinant expression vector to mimic the endogenous expression of B7-1.

IV. Uses for the Isolated Nucleic Acid Molecules of the Invention

A. Probes

The isolated nucleic acids of the invention are useful for constructing nucleotide probes for use in detecting nucleotide sequences in biological materials, such as cell extracts, or directly in cells (e.g., by *in situ* hybridization). A nucleotide probe can be labeled with a radioactive element which provides for an adequate signal as a means for detection and has sufficient half-life to be useful for detection, such as ³²P, ³H, ¹⁴C or the like. Other materials which can be used to label the probe include antigens that are recognized by a specific labeled antibody, fluorescent compounds, enzymes and chemiluminescent compounds. An appropriate label can be selected with regard to the rate of hybridization and binding of the probe to the nucleotide sequence to be detected and the amount of nucleotide available for hybridization. The isolated nucleic acids of the invention, or oligonucleotide fragments thereof, can be used as suitable probes for a variety of hybridization procedures well known to those skilled in the art. The isolated nucleic acids of the invention enable one to determine whether a cell expresses an alternatively spliced form of a T cell costimulatory

molecule. For example, mRNA can be prepared from a sample of cells to be examined and the mRNA can be hybridized to an isolated nucleic acid encompassing a nucleotide sequence encoding all or a portion of an alternative cytoplasmic domain of a T cell costimulatory molecule (e.g., SEQ ID NO: 1) to detect the expression of the alternative cytoplasmic domain form of the costimulatory molecule in the cells. Furthermore, the isolated nucleic acids of the invention can be used to design oligonucleotide primers, e.g. PCR primers, which allow one to detect the expression of an alternatively spliced form of a T cell costimulatory molecule. Preferably, this oligonucleotide primer spans a novel exon junction created by alternative splicing and thus can only amplify cDNAs encoding this alternatively spliced form. For example, an oligonucleotide primer which spans exon 4 and exon 6 of murine B7-1 can be used to distinguish between the expression of a first cytoplasmic domain form of mB7-1 (i.e., encoded by exons 1-2-3-4-5) and expression of an alternative second cytoplasmic domain form of a costimulatory molecule (i.e., encoded by exons 1-2-3-4-6) (e.g., see Example 2).

The probes of the invention can be used to detect an alteration in the expression of an alternatively spliced form of a T cell costimulatory molecule, such as in a disease state. For example, detection of a defect in the expression of an alternatively spliced form of a T cell costimulatory molecule that is associated with an immunodeficiency disorder can be used to diagnose the disorder (i.e., the probes of the invention can be used for diagnostic purposes). Many congenital immunodeficiency diseases result from lack of expression of a cell-surface antigen important for interactions between T cells and antigen presenting cells. For example, the bare lymphocyte syndrome results from lack of expression of MHC class II antigens (see e.g., Rijkers, G.T. et al. (1987) *J. Clin. Immunol.* 7:98-106; Hume, C.R. et al. (1989) *Hum. Immunol.* 25:1-11)) and X-linked hyperglobulinemia results from defective expression of the ligand for CD40 (gp39) (see e.g. Korthauer, U et al. (1993) *Nature* 361:541; Aruffo, A. et al. (1993) *Cell* 72:291-300). An immunodeficiency disorder which results from lack of expression of an alternatively spliced form of a T cell costimulatory molecule can be diagnosed using a probe of the invention. For example, a disorder resulting from the lack of expression of the Cyt II form of B7-1 can be diagnosed in a patient based upon the inability of a probe which detects this form of B7-1 (e.g., an oligonucleotide spanning the junction of exon 4 and exon 6) to hybridize to mRNA in cells from the patient (e.g., by RT-PCR or by Northern blotting).

B. Recombinant Expression Vectors

An isolated nucleic acid of the invention can be incorporated into an expression vector (i.e., a recombinant expression vector) to direct expression of a novel structural form of a T cell costimulatory molecule encoded by the nucleic acid. The recombinant expression vectors are suitable for transformation of a host cell, and include a nucleic acid (or fragment thereof) of the invention and a regulatory sequence, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid. Operatively linked is

intended to mean that the nucleic acid is linked to a regulatory sequence in a manner which allows expression of the nucleic acid. Regulatory sequences are art-recognized and are selected to direct expression of the desired protein in an appropriate host cell. Accordingly, the term regulatory sequence includes promoters, enhancers and other expression control elements. Such regulatory sequences are known to those skilled in the art or are described in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transfected and/or the type of protein desired to be expressed. Such expression vectors can be used to transfect cells to thereby produce proteins or peptides encoded by nucleic acids as described herein.

The recombinant expression vectors of the invention can be designed for expression of encoded proteins in prokaryotic or eukaryotic cells. For example, proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus), yeast cells or mammalian cells. Other suitable host cells can be found in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Expression in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids usually to the amino terminus of the expressed target gene. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the target recombinant protein; and 3) to aid in the purification of the target recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the target recombinant protein to enable separation of the target recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase, maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Inducible non-fusion prokaryotic expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). In pTrc, target gene expression relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. In pET11d, expression of inserted target genes relies on transcription from the T7 gn10-lac 0 fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacterial strain with an impaired capacity to proteolytically cleave the

recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector (e.g., a nucleic acid of the invention) so that the individual codons for each amino acid would be those preferentially utilized in highly expressed *E. coli* proteins (Wada *et al.*, (1992) *Nuc. Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques and are encompassed by the invention.

Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari. *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Baculovirus vectors available for expression of proteins in cultured insect cells (SF 9 cells) include the pAc series (Smith *et al.*, (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow, V.A., and Summers, M.D., (1989) *Virology* 170:31-39).

Expression of alternatively spliced forms of T cell costimulatory molecules in mammalian cells is accomplished using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B., (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987), *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. The recombinant expression vector can be designed such that expression of the nucleic acid occurs preferentially in a particular cell type. In this situation, the expression vector's control functions are provided by regulatory sequences which allow for preferential expression of a nucleic acid contained in the vector in a particular cell type, thereby allowing for tissue or cell specific expression of an encoded protein.

The recombinant expression vectors of the invention can be a plasmid or virus, or viral portion which allows for expression of a nucleic acid introduced into the viral nucleic acid. For example, replication defective retroviruses, adenoviruses and adeno-associated viruses can be used. The recombinant expression vectors can be introduced into a host cell, e.g. *in vitro* or *in vivo*. A host cell line can be used to express a protein of the invention. Furthermore, introduction of a recombinant expression vector of the invention into a host cell can be used for therapeutic purposes when the host cell is defective in expressing the novel structural form of the T cell costimulatory molecule. For example, in a recombinant expression vector of the invention can be used for gene therapy purposes in a patient with an immunodeficiency disorder resulting from lack of expression of a novel structural form of a T cell costimulatory molecule.

C. Host Cells

The invention further provides a host cell transfected with a recombinant expression vector of the invention. The term "host cell" is intended to include prokaryotic and

eukaryotic cells into which a recombinant expression vector of the invention can be introduced. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (e.g., a vector) into a cell by one of a number of possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. Nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate co-precipitation, DEAE-dextran-mediated transfection, lipofectin, electroporation or microinjection. Suitable methods for transforming and transfecting host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory handbooks.

The number of host cells transfected with a recombinant expression vector of the invention by techniques such as those described above will depend upon the type of recombinant expression vector used and the type of transfection technique used. Typically, plasmid vectors introduced into mammalian cells are integrated into host cell DNA at only a low frequency. In order to identify these integrants, a gene that contains a selectable marker (i.e., resistance to antibiotics) can be introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to certain drugs, such as G418 and hygromycin. Selectable markers can be introduced on a separate vector (e.g., plasmid) from the nucleic acid of interest or, preferably, are introduced on the same vector (e.g., plasmid). Host cells transformed with one or more recombinant expression vectors containing a nucleic acid of the invention and a gene for a selectable marker can be identified by selecting for cells using the selectable marker. For example, if the selectable marker encoded a gene conferring neomycin resistance, transformant cells can be selected with G418. Cells that have incorporated the selectable marker gene will survive, while the other cells die.

Preferably, the novel cytoplasmic domain form of the T cell costimulatory molecule is expressed on the surface of a host cell (e.g., on the surface of a mammalian cell). This is accomplished by using a recombinant expression vector encoding extracellular domains (e.g., signal peptide, V-like and/or C-like domains), transmembrane and cytoplasmic domains of the T cell costimulatory molecule with appropriate regulatory sequences (e.g., a signal sequence) to allow for surface expression of the translated protein.

In one embodiment, a host cell is transfected with a recombinant expression vector encoding a second, novel cytoplasmic domain form of a T cell costimulatory molecule. In a preferred embodiment, the host cell does not express the first (i.e., previously disclosed) cytoplasmic domain form of the costimulatory molecule. For example, a host cell which does not express a form of murine B7-1 containing Cyt I can be transfected with a recombinant expression vector encoding a form of murine B7-1 containing Cyt II. Such a host cell will thus exclusively express the form of B7-1 containing Cyt II. This type of host

cell is useful for studying signaling events and/or immunological responses which are mediated by the Cyt II domain rather than the Cyt I domain of B7-1. For example, one type of cell which can be used to create a host cell which exclusively expresses the Cyt II-form of murine B7-1 is a non-murine cell, since the non-murine cell does not express murine B7-1.

- 5 Preferably, the non-murine cell also does not express other costimulatory molecules (e.g., COS cells can be used). Alternatively, a mouse cell which does not express the Cyt-I form of murine B7-1 can be used. For example, a recombinant expression vector of the invention can be introduced into NIH 3T3 fibroblast cells (which are B7-1 negative) or into cells derived from a mutant mouse in which the endogenous B7-1 gene has been disrupted and thus which
10 does not natively express any form of B7-1 molecule (i.e., into cells derived from a "B7-1 knock-out" mouse, such as that described in Freeman, G.J. et al. (1993) *Science* 262:907-909).

In another embodiment, the host cell transfected with a recombinant expression vector encoding a novel structural form of a T cell costimulatory molecule is a tumor cell.

- 15 Expression of the Cyt-I form of murine B7-1 on the surface of B7-1 negative murine tumor cells has been shown to induce T cell mediated specific immunity against the tumor cells accompanied by tumor rejection and prolonged protection to tumor challenge in mice (see Chen, L., et al. (1992) *Cell* 71, 1093-1102; Townsend, S.E. and Allison, J.P. (1993) *Science* 259, 368-370; Baskar, S., et al. (1993) *Proc. Natl. Acad. Sci.* 90, 5687-5690). Similarly,
20 expression of novel structural forms of costimulatory molecules on the surface of a tumor cell may be useful for increasing the immunogenicity of the tumor cell. For example, tumor cells obtained from a patient can be transfected *ex vivo* with a recombinant expression vector of the invention, e.g., encoding an alternative cytoplasmic domain form of a costimulatory molecule, and the transfected tumor cells can then be returned to the patient. Alternatively,
25 gene therapy techniques can be used to target a tumor cell for transfection *in vivo*. Additionally, the tumor cell can also be transfected with recombinant expression vectors encoding other proteins to be expressed on the tumor cell surface to increase the immunogenicity of the tumor cell. For example, the Cyt-I form of B7-1, B7-2, MHC molecules (e.g., class I and/or class II) and/or adhesion molecules can be expressed on the
30 tumor cells in conjunction with the Cyt-II form of B7-1.

D. Anti-Sense Nucleic Acid Molecules

- The isolated nucleic acid molecules of the invention can also be used to design anti-sense nucleic acid molecules, or oligonucleotide fragments thereof, that can be used to
35 modulate the expression of alternative forms of T cell costimulatory molecules. An anti-sense nucleic acid comprises a nucleotide sequence which is complementary to a coding strand of a nucleic acid, e.g. complementary to an mRNA sequence, constructed according to the rules of Watson and Crick base pairing, and can hydrogen bond to the coding strand of the nucleic acid. The hydrogen bonding of an antisense nucleic acid molecule to an mRNA

transcript can prevent translation of the mRNA transcript and thus inhibit the production of the protein encoded therein. Accordingly, an anti-sense nucleic acid molecule can be designed which is complementary to a nucleotide sequence encoding a novel structural domain of a T cell costimulatory molecule to inhibit production of that particular structural form of the T cell costimulatory molecule. For example, an anti-sense nucleic acid molecule can be designed which is complementary to a nucleotide sequence encoding the Cyt-II form of murine B7-1 and used to inhibit the expression of this form of the costimulatory molecule.

An anti-sense nucleic acids molecule, or oligonucleotide fragment thereof, can be constructed by chemical synthesis and enzymatic ligation reactions using procedures known in the art. The anti-sense nucleic acid or oligonucleotide can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the ant-sense and sense nucleic acids e.g. phosphorothioate derivatives and acridine substituted nucleotides can be used. Alternatively, the anti-sense nucleic acids and oligonucleotides can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an anti-sense orientation (i.e. nucleic acid transcribed from the inserted nucleic acid will be of an anti-sense orientation to a target nucleic acid of interest). The anti-sense expression vector is introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which anti-sense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using anti-sense genes see Weintraub, H. et al., "Antisense RNA as a molecular tool for genetic analysis", *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

E. Non-Human Transgenic and Homologous Recombinant Animals

The isolated nucleic acids of the invention can further be used to create a non-human transgenic animal. A transgenic animal is an animal having cells that contain a transgene, wherein the transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic, stage. A transgene is a DNA molecule which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. Accordingly, the invention provides a non-human transgenic animal which contains cells transfected to express an alternative form of a T cell costimulatory molecule. Preferably, the non-human animal is a mouse. A transgenic animal can be created, for example, by introducing a nucleic acid encoding the protein (typically linked to appropriate regulatory elements, such as a tissue-specific enhancer) into the male pronuclei of a fertilized oocyte, e.g., by microinjection, and allowing the oocyte to develop in a pseudopregnant female foster animal. For example, a transgenic animal (e.g., a mouse) which expresses an mB7-1 protein containing a novel

cytoplasmic domain (e.g. Cyt-II) can be made using the isolated nucleic acid shown in SEQ ID NO: 1 or SEQ ID NO: 3. Alternatively, a transgenic animal (e.g., a mouse) which expresses an mB7-2 protein containing an alternative signal peptide domain can be made using the isolated nucleic acid shown in SEQ ID NO: 12. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. These isolated nucleic acids can be linked to regulatory sequences which direct the expression of the encoded protein one or more particular cell types. Methods for generating transgenic animals, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009 and Hogan, B. et al., (1986) *A Laboratory Manual*, Cold Spring Harbor, New York, Cold Spring Harbor Laboratory. A transgenic founder animal can be used to breed additional animals carrying the transgene.

The isolated nucleic acids of the invention can further be used to create a non-human homologous recombinant animal. The term "homologous recombinant animal" as used herein is intended to describe an animal containing a gene which has been modified by homologous recombination. The homologous recombination event may completely disrupt the gene such that a functional gene product can no longer be produced (often referred to as a "knock-out" animal) or the homologous recombination event may modify the gene such that an altered, although still functional, gene product is produced. Preferably, the non-human animal is a mouse. For example, an isolated nucleic acid of the invention can be used to create a homologous recombinant mouse in which a recombination event has occurred in the B7-1 gene at an exon encoding a cytoplasmic domain such that this exon is altered (e.g., exon 5 or exon 6 is altered). Homologous recombinant mice can thus be created which express only the Cyt I or Cyt II domain form of B7-1. Accordingly, the invention provides a non-human knock-out animal which contains a gene encoding a B7-1 protein wherein an exon encoding a novel cytoplasmic domain is disrupted or altered.

To create an animal with homologously recombined nucleic acid, a vector is prepared which contains the DNA sequences which are to replace the endogenous DNA sequences, flanked by DNA sequences homologous to flanking endogenous DNA sequences (see for example Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected (see for example Li, E. et al. (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see for example Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA.

V. Isolated Novel Forms of Costimulatory Molecules

The invention further provides isolated T cell costimulatory molecules encoded by the nucleic acids of the invention. These molecules have a novel structural form, either
5 containing a novel structural domain or having a structural domain deleted or added. The term "isolated" refers to a T cell costimulatory molecule, e.g., a protein, substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. In one embodiment, the novel T cell costimulatory molecule is a B7-1 protein. In another embodiment, the novel
10 T cell costimulatory molecule is a B7-2 protein.

A. Proteins with a Novel Cytoplasmic Domain

One aspect of the invention pertains to a T cell costimulatory molecule which includes at least one novel cytoplasmic domain. In one embodiment, the invention provides a
15 protein which binds to CD28 and/or CTLA4 and has an amino acid sequence derived from amino acid sequences encoded by at least one T cell costimulatory molecule gene. In this embodiment, the protein comprises a contiguous amino acid sequence represented by a formula A-B-C-D-E, wherein

20 A, which may or may not be present, comprises an amino acid sequence of a signal peptide domain,

B comprises an amino acid sequence of an immunoglobulin variable region-like domain encoded by at least one exon of a T cell costimulatory molecule gene,

25 C comprises an amino acid sequence of an immunoglobulin constant region-like domain encoded by at least one exon of a T cell costimulatory molecule gene,

D comprises an amino acid sequence of a transmembrane domain encoded by at least one exon of a T cell costimulatory molecule gene, and

E comprises an amino acid sequence of a cytoplasmic domain encoded by at least one exon of a T cell costimulatory molecule gene,

30

with the proviso that E does not comprise an amino acid sequence of a cytoplasmic domain selected from the group consisting of SEQ ID NO: 26 (mB7-1), SEQ ID NO: 28 (hB7-1), SEQ ID NO: 30 (mB7-2), and SEQ ID NO: 32 (hB7-2).

In the formula, A, B, C, D, and E are contiguous amino acid residues linked by amide
35 bonds from an N-terminus to a C-terminus. According to the formula, A can be an amino acid sequence of a signal peptide domain of a heterologous protein which efficiently expresses transmembrane or secreted proteins, such as the oncostatin M signal peptide. Preferably, A, if present, comprises an amino acid sequence of a signal peptide domain encoded by at least one exon of a T cell costimulatory molecule gene. In one preferred

embodiment, the isolated protein is a B7-1 or a B7-2 protein. E preferably comprises an amino acid sequence of a murine B7-1 cytoplasmic domain having an amino acid sequence shown in SEQ ID NO: 5 (i.e., the amino acid sequence of the cytoplasmic domain encoded by the novel exon 6 of the invention).

5 Another embodiment of the invention provides an isolated protein which binds CD28 or CTLA4 and is encoded by a T cell costimulatory molecule gene having at least one first exon encoding a first cytoplasmic domain and at least one second exon encoding a second cytoplasmic domain. The at least one first cytoplasmic domain comprises an amino acid sequence selected from the group consisting of amino acid sequence of SEQ ID NO:26
10 (mB7-1), SEQ ID NO:28 (hB7-1), SEQ ID NO:30 (mB7-2) and SEQ ID NO:32 (hB7-2). In this embodiment, the protein includes an amino acid sequence comprising at least one second cytoplasmic domain. Preferably, the protein does not include an amino acid sequence comprising a first cytoplasmic domain.

Preferred proteins which bind CD28 and/or CTLA4 are derived from B7-1 and B7-2.
15 In a particularly preferred embodiment, the invention provides an isolated protein which binds CD28 or CTLA4 and has a novel cytoplasmic domain comprising an amino acid sequence shown in SEQ ID NO: 2.

A. Proteins with a Novel Signal Peptide Domain

20 In yet another aspect of the invention, T cell costimulatory molecules which include at least one novel signal peptide domain are provided. In one embodiment, the isolated protein binds to CD28 or CTLA4 and has an amino acid sequence derived from amino acid sequences encoded by at least one T cell costimulatory molecule gene. In this embodiment, the protein comprises a contiguous amino acid sequence represented by a formula A-B-C-D-
25 E, wherein

A comprises an amino acid sequence of a signal peptide domain encoded by at least one exon of a T cell costimulatory molecule gene,

30 B comprises an amino acid sequence of an immunoglobulin variable region-like domain encoded by at least one exon of a T cell costimulatory molecule gene,

C comprises an amino acid sequence of an immunoglobulin constant region-like domain encoded by at least one exon of a T cell costimulatory molecule gene,

35 D, which may or may not be present, comprises an amino acid sequence of a transmembrane domain encoded by at least one exon of a T cell costimulatory molecule gene, and

E, which may or may not be present, comprises an amino acid sequence of a cytoplasmic domain encoded by at least one exon of a T cell costimulatory molecule gene,

with the proviso that A not comprise an amino acid sequence of a signal peptide domain selected from the group consisting of SEQ ID NO: 34 (mB7-1), SEQ ID NO: 36 (hB7-1), SEQ ID NO: 38 (mB7-2), SEQ ID NO: 40 (hB7-2), SEQ ID NO: 42 (hB7-2).

5 In the formula, A, B, C, D, and E are contiguous amino acid residues linked by amide bonds from an N-terminus to a C-terminus. To produce a soluble form of the T cell costimulatory molecule D, which comprises an amino acid sequence of a transmembrane domain and E, which comprises an amino acid sequence of a cytoplasmic domain may not be present in the molecule. Preferably, A comprises an amino acid sequence of a novel signal peptide domain shown in SEQ ID NO: 15.

10 In another embodiment of the invention, the isolated protein which binds CD28 or CTLA4 is encoded by a T cell costimulatory molecule gene having at least one first exon encoding a first signal peptide domain and at least one second exon encoding a second signal peptide domain. The at least one first signal peptide domain comprises an amino acid sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:34
15 (mB7-1), SEQ ID NO:36 (hB7-1), SEQ ID NO:38 (mB7-2) and SEQ ID NO:40 (hB7-2) and SEQ ID NO:42 (hB7-2). In this embodiment, the protein includes an amino acid sequence comprising at least one second signal peptide domain. Preferably, the protein does not include an amino acid sequence comprising a first signal peptide domain.

Preferred proteins which bind CD28 and/or CTLA4 are derived from B7-1 and B7-2.
20 In a particularly preferred embodiment, the invention features a murine B7-2 protein comprising an amino acid sequence shown in SEQ ID NO: 13.

C. Isolated Proteins with Structural Domains Deleted or Added

This invention also features costimulatory molecules which have at least one
25 structural domain deleted. In one embodiment, the structural form has at least one IgV-like domain deleted. Accordingly, in one embodiment, the isolated protein has an amino acid sequence derived from amino acid sequences encoded by at least one T cell costimulatory molecule gene and comprises a contiguous amino acid sequence represented by a formula A-B-C-D, wherein

30 A, which may or may not be present, comprises an amino acid sequence of a signal peptide domain encoded by at least one exon of a T cell costimulatory molecule gene,

B comprises an amino acid sequence of an immunoglobulin constant region-like domain encoded by at least one exon of a T cell costimulatory molecule gene, and
35

C comprises an amino acid sequence of a transmembrane domain encoded by at least one exon of a T cell costimulatory molecule gene, and

D comprises an amino acid sequence of a cytoplasmic domain encoded by at least one exon of a T cell costimulatory molecule gene.

In the formula, A, B, C and D are contiguous amino acid residues linked by amide bonds from an N-terminus to a C-terminus. In a preferred embodiment, an isolated murine B7-1 protein having an IgV-like domain deleted comprises an amino acid sequence shown in SEQ ID NO: 9 (utilizing Cyt I of mB7-1). Alternatively, an isolated murine B7-1 protein having an IgV-like domain deleted comprises an amino acid sequence shown in SEQ ID NO: 11 (utilizing Cyt II of mB7-1).

In another embodiment, the structural form of the T cell costimulatory molecule has at least one IgC-like domain deleted. Accordingly, in one embodiment, the isolated protein has an amino acid sequence derived from amino acid sequences encoded by at least one T cell costimulatory molecule gene and comprises a contiguous amino acid sequence represented by a formula A-B-C-D, wherein

A, which may or may not be present, comprises an amino acid sequence of a signal peptide domain encoded by at least one exon of a T cell costimulatory molecule gene,

B comprises an amino acid sequence of an immunoglobulin variable region-like domain encoded by at least one exon of a T cell costimulatory molecule gene, and

C comprises an amino acid sequence of a transmembrane domain encoded by at least one exon of a T cell costimulatory molecule gene, and

D comprises an amino acid sequence of a cytoplasmic domain encoded by at least one exon of a T cell costimulatory molecule gene.

In the formula, A, B, C and D are contiguous amino acid residues linked by amide bonds from an N-terminus to a C-terminus. In a preferred embodiment, an isolated murine B7-1 protein having an IgC-like domain deleted comprises an amino acid sequence shown in SEQ ID NO: 63 (utilizing Cyt I of mB7-1). Alternatively, an isolated murine B7-1 protein having an IgC-like domain deleted comprises an amino acid sequence shown in SEQ ID NO: 65 (utilizing Cyt II of mB7-1).

The proteins of the invention can be isolated by expression of the molecules (e.g., proteins or peptide fragments thereof) in a suitable host cell using techniques known in the art. Suitable host cells include prokaryotic or eukaryotic organisms or cell lines, for example, yeast, *E. coli* and insect cells. The recombinant expression vectors of the invention, described above, can be used to express a costimulatory molecule in a host cell in order to isolate the protein. The invention provides a method of preparing an isolated protein of the invention comprising introducing into a host cell a recombinant expression vector encoding the protein, allowing the protein to be expressed in the host cell and isolating the protein. Proteins can be isolated from a host cell expressing the protein according to standard procedures of the art, including ammonium sulfate precipitation, fractionation column

chromatography (e.g. ion exchange, gel filtration, electrophoresis, affinity chromatography, etc.) and ultimately, crystallization (see generally, "Enzyme Purification and Related Techniques", *Methods in Enzymology*, 22, 233-577 (1971)).

Alternatively, the costimulatory molecules of the invention can be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, J. Am. Chem. Assoc. 85:2149-2154) or synthesis in homogeneous solution (Houbenweyl, 1987, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart).

VI. Uses For the Novel T Cell Costimulatory Molecules of the Invention

A. Costimulation

The novel T cell costimulatory molecules of the invention can be used to trigger a costimulatory signal in T cells. When membrane-bound or in a multivalent form, a T cell costimulatory molecule can trigger a costimulatory signal in a T cell by allowing the costimulatory molecule to interact with its receptor (e.g., CD28) on T cells in the presence of a primary activation signal. A novel T cell costimulatory molecule of the invention can be obtained in membrane-bound form by expressing the molecule in a host cell (e.g., by transfecting the host cell with a recombinant expression vector encoding the molecule). To be expressed on the surface of a host cell, the T cell costimulatory molecule should include extracellular domains (i.e., signal peptide, which may or may not be present in the mature protein, IgV-like and IgC-like domains), a transmembrane domain and a cytoplasmic domain. To trigger a costimulatory signal, T cells are contacted with the cell expressing the costimulatory molecule, preferably together with a primary activation signal (e.g., MHC-associated antigenic peptide, anti-CD3 antibody, phorbol ester etc.). Activation of the T cell can be assayed by standard procedures, for example by measuring T cell proliferation or cytokine production.

The novel T cell costimulatory molecules of the invention can also be used to inhibit or block a costimulatory signal in T cells. A soluble form of a T cell costimulatory molecule can be used to competitively inhibit the interaction of membrane-bound costimulatory molecules with their receptor (e.g., CD28 and/or CTLA4) on T cells. A soluble form of a T cell costimulatory molecule can be expressed in host cell line such that it is secreted by the host cell line and can then be purified. The soluble costimulatory molecule contains extracellular domains (i.e., signal peptide, which may or may not be present in the mature protein, IgV-like and IgC-like domains) but does not contain a transmembrane or cytoplasmic domain. The soluble form of the T cell costimulatory molecule can also be in the form of a fusion protein, e.g. an immunoglobulin fusion protein wherein the extracellular portion of the costimulatory molecule is fused to an immunoglobulin constant region. A soluble form of a

T cell costimulatory molecule can be used to inhibit a costimulatory signal in T cells by contacting the T cells with the soluble molecule.

B. Antibodies

5 A novel structural form of a T cell costimulatory molecule of the invention can be used to produce antibodies directed against the costimulatory molecule. Conventional methods can be used to prepare the antibodies. For example, to produce polyclonal antibodies, a mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with a costimulatory molecule, or an immunogenic portion thereof, which elicits an antibody
10 response in the mammal. Techniques for conferring immunogenicity on a protein include conjugation to carriers or other techniques well known in the art. For example, the protein can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies.
15 Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera.

In addition to polyclonal antisera, the novel costimulatory molecules of the invention can be used to raise monoclonal antibodies. To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused with
20 myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art. For example, the hybridoma technique originally developed by Kohler and Milstein (*Nature* 256, 495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., *Immunol. Today* 4, 72 (1983)), the EBV-hybridoma technique to produce human
25 monoclonal antibodies (Cole et al. *Monoclonal Antibodies in Cancer Therapy* (1985) Allen R. Bliss, Inc., pages 77-96), and screening of combinatorial antibody libraries (Huse et al., *Science* 246, 1275 (1989)). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the protein or portion thereof and monoclonal antibodies isolated.

30 The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with an alternative cytoplasmic domain of a costimulatory molecule. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab')₂ fragments can be generated by treating antibody with pepsin. The resulting F(ab')₂ fragment
35 can be treated to reduce disulfide bridges to produce Fab' fragments.

Chimeric and humanized antibodies are also within the scope of the invention. It is expected that chimeric and humanized antibodies would be less immunogenic in a human subject than the corresponding non-chimeric antibody. A variety of approaches for making chimeric antibodies, comprising for example a non-human variable region and a human

constant region, have been described. See, for example, Morrison et al., *Proc. Natl. Acad. Sci. U.S.A.* 81, 6851 (1985); Takeda et al., *Nature* 314, 452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom Patent GB 2177096B. Additionally, a chimeric antibody can be further "humanized" antibodies such that parts of the variable regions, especially the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such altered immunoglobulin molecules may be made by any of several techniques known in the art, (e.g., Teng et al., *Proc. Natl. Acad. Sci. U.S.A.*, 80, 7308-7312 (1983); Kozbor et al., *Immunology Today*, 4, 7279 (1983); Olsson et al., *Meth. Enzymol.*, 92, 3-16 (1982)), and are preferably made according to the teachings of PCT Publication WO92/06193 or EP 0239400. Humanized antibodies can be commercially produced by, for example, Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.

Another method of generating specific antibodies, or antibody fragments, reactive against an alternative cytoplasmic domain of the invention is to screen phage expression libraries encoding immunoglobulin genes, or portions thereof, with proteins produced from the nucleic acid molecules of the present invention (e.g., with all or a portion of the amino acid sequence of SEQ ID NO: 7). For example, complete Fab fragments, V_H regions and F_V regions can be expressed in bacteria using phage expression libraries. See for example Ward et al., *Nature* 341, 544-546: (1989); Huse et al., *Science* 246, 1275-1281 (1989); and McCafferty et al. *Nature* 348, 552-554 (1990).

In a preferred embodiment, the invention provides an antibody which binds to a novel structural domain of a T cell costimulatory molecule provided by the invention. Such antibodies, and uses therefor, are described in greater detail below in subsection VI, part B.

C. Screening Assays

A T cell costimulatory molecule of the invention containing a novel cytoplasmic domain can be used in a screening assay to identify components of the intracellular signal transduction pathway induced in antigen presenting cells upon binding of the T cell costimulatory molecule to its receptor on a T cell. In addition to triggering a costimulatory signal in T cells, engagement of the costimulatory molecule with a receptor on T cells is likely to deliver distinct signals to the antigen presenting cell (i.e., the cell expressing the T cell costimulatory molecule), e.g. through the cytoplasmic domain. Signals delivered through a novel cytoplasmic domain of the invention may be of particular importance in the thymus, e.g., during positive selection of T cells during development, since structural forms of costimulatory molecules comprising a novel cytoplasmic domain are preferentially expressed in the thymus. A host cell which exclusively expresses a Cyt-II form of a costimulatory molecule (e.g., mB7-1) is especially useful for elucidating such intracellular signal transduction pathways. For example, a host cell which expresses only a Cyt-II form of the

costimulatory molecule can be stimulated through the costimulatory molecule, e.g., by crosslinking the costimulatory molecules on the cell surface with an antibody, and intracellular signals and/or other cellular changes (e.g., changes in surface expression of proteins etc.) induced thereupon can be identified.

5 Additionally, an isolated T cell costimulatory molecule of the invention comprising a novel cytoplasmic domain can be used in methods of identifying other molecules (e.g., proteins) which interact with (i.e., bind to) the costimulatory molecule using standard *in vitro* assays (e.g., incubating the isolated costimulatory molecule with a cellular extract and determining by immunoprecipitation if any molecules within the cellular extract bind to the
10 costimulatory molecule). It is of particular interest to identify molecules which can interact with the novel cytoplasmic domain since such molecules may also be involved in intracellular signaling. For example, it is known that the cytoplasmic domains of many cell-surface receptors can interact intracellularly with other members of the signal transduction machinery, e.g., tyrosine kinases.

15 The invention further provides a method for screening agents to identify an agent which upregulates or downregulates expression of a novel structural domain form of a T cell costimulatory molecule. The method involves contacting a cell which expresses or can be induced to express a T cell costimulatory molecule with an agent to be tested and determining expression of a novel structural domain form of the T cell costimulatory molecule by the cell.
20 The term "upregulates" encompasses inducing the expression of a novel form of a T cell costimulatory molecule by a cell which does not constitutively express such a molecule or increasing the level of expression of a novel form of a T cell costimulatory molecule by a cell which already expresses such a molecule. The term "downregulates" encompasses decreasing or eliminating expression of an a novel form of a T cell costimulatory molecule by
25 a cell which already expresses such a molecule. The term "agent" is intended to include molecules which trigger an upregulatory or downregulatory response in a cell. For example, an agent can be a small organic molecule, a biological response modifier (e.g., a cytokine) or a molecule which can crosslink surface structures on the cell (e.g., an antibody). For example, expression of the a novel cytoplasmic domain form of the T cell costimulatory
30 molecule by the cell can be determined by detecting an mRNA transcript encoding the novel cytoplasmic domain form of the T cell costimulatory molecule in the cell. For example, mRNA from the cell can be reverse transcribed and used as a template in PCR reactions utilizing PCR primers which can distinguish between a Cyt I cytoplasmic domain form and a novel Cyt II cytoplasmic domain form of the T cell costimulatory molecule (see e.g.,
35 Example 2). Alternatively, a novel cytoplasmic domain-containing T cell costimulatory molecule can be detected in the cell using an antibody directed against the novel cytoplasmic domain (e.g., by immunoprecipitation or immunohistochemistry). A preferred T cell costimulatory molecule for use in the method is B7-1. Cell types which are known to express the Cyt-I form of B7-1, or which can be induced to express the Cyt-I form of B7-1, include B

lymphocytes, T lymphocytes and monocytes. Such cell types can be screened with agents according to the method of the invention to identify an agent which upregulates or downregulates expression of the Cyt-II form of B7-1.

5 VI. Isolated Novel Structural Domains of T Cell Costimulatory Molecules and Uses Therefor

Another aspect of the invention pertains to isolated nucleic acids encoding novel structural domains of T cell costimulatory molecules provided by the invention. In one embodiment, the structural domain encoded by the nucleic acid is a cytoplasmic domain. A preferred nucleic acid encoding a novel cytoplasmic domain comprises a nucleotide sequence
10 shown in SEQ ID NO: 4. In another embodiment, the structural domain encoded by the nucleic acid is a signal peptide domain. A preferred nucleic acid encoding a novel signal peptide domain comprises a nucleotide sequence shown in SEQ ID NO: 14.

The invention also provides isolated polypeptides corresponding to novel structural domains of T cell costimulatory molecules, encoded by nucleic acids of the invention. In one
15 embodiment, the structural domain is a cytoplasmic domain. A preferred novel cytoplasmic domain comprises an amino acid sequence shown in SEQ ID NO: 5. In another embodiment, the structural domain is a signal peptide domain. A preferred novel signal peptide domain comprises an amino acid sequence shown in SEQ ID NO: 15.

The uses of the novel structural domains of the invention include the creation of
20 chimeric proteins. The domains can further be used to raise antibodies specifically directed against the domains.

A. Chimeric Proteins

The invention provides a fusion protein comprised of two peptides, a first peptide and
25 a second peptide, wherein the second peptide is a novel structural domain of a T cell costimulatory molecule provided by the invention. In one embodiment, the novel structural domain is a cytoplasmic domain, preferably comprising an amino acid sequence shown in SEQ ID NO: 5. In another embodiment, the novel structural domain is a signal peptide domain, preferably comprising an amino acid sequence shown in SEQ ID NO: 15. For
30 example, a fusion protein can be made which contains extracellular and transmembrane portions from a protein other than murine B7-1 and which contains a novel cytoplasmic domain (e.g., Cyt-II) of murine B7-1. This type of fusion protein can be made using standard recombinant DNA techniques in which a nucleic acid molecule encoding the cytoplasmic domain (e.g., SEQ ID NO:4) is linked in-frame to the 3' end of a nucleic acid molecule
35 encoding the extracellular and transmembrane domains of the protein. The recombinant nucleic acid molecule can be incorporated into an expression vector and the encoded fusion protein can be expressed by standard techniques, e.g., by transfecting the recombinant expression vector into an appropriate host cell and allowing expression of the fusion protein.

A fusion protein of the invention, comprising a first peptide fused to a second peptide comprising a novel cytoplasmic domain of the invention, can be used to transfer the signal transduction function of the novel cytoplasmic domain to another protein. For example, a novel cytoplasmic domain of B7-1 (e.g., Cyt-II) can be fused to the extracellular and transmembrane domains of another protein (e.g., an immunoglobulin protein, a T cell receptor protein, a growth factor receptor protein etc.) and the fusion protein can be expressed in a host cell by standard techniques. The extracellular domain of the fusion protein can be crosslinked (e.g., by binding of a ligand or antibody to the extracellular domain) to generate an intracellular signal(s) mediated by the novel cytoplasmic domain.

Additionally, a fusion protein of the invention can be used in methods of identifying and isolating other molecules (e.g., proteins) which can interact intracellularly (i.e., within the cell cytoplasm) with a novel cytoplasmic domain of the invention. One approach to identifying molecules which interact intracellularly with the cytoplasmic domain of a cell-surface receptor is to metabolically label cells which express the receptor, immunoprecipitate the receptor, usually with an antibody against the extracellular domain of the receptor, and identify molecules which are co-immunoprecipitated along with the receptor. In the case of mB7-1, however, the cells which have been found to express the naturally-occurring Cyt-II form of B7-1 have also been found to express the naturally-occurring Cyt-I form of B7-1 (e.g., thymocytes, see Example 2). Thus, immunoprecipitation with an antibody against the extracellular domain of mB7-1 would immunoprecipitate both forms of the protein since the extracellular domain is common to both the Cyt-I and Cyt-II containing forms. Thus, molecules which interact with either Cyt-I or Cyt-II would be co-immunoprecipitate. A fusion protein comprising a non-B7-1 extracellular domain (to which an antibody can bind), a transmembrane domain (derived either from the non-B7-1 molecule or from B7-1) and a B7-1 alternative cytoplasmic domain (e.g., Cyt-II) can be constructed and expressed in a host cell which naturally expresses the Cyt-II form of B7-1. The antibody directed against the "heterologous" extracellular domain of the fusion protein can then be used to immunoprecipitate the fusion protein and to co-immunoprecipitate any other proteins which interact intracellularly with the novel cytoplasmic domain.

B. Antibodies

An antibody which binds to a novel structural domain of the invention can be prepared by using the domain, or a portion thereof, as an immunogen. Polyclonal antibodies or monoclonal antibodies can be prepared by standard techniques described above. In a preferred approach, peptides comprising amino acid sequences of the domain are used as immunogens, e.g. overlapping peptides encompassing the amino acid sequence of the domain. For example, polyclonal antisera against a novel cytoplasmic domain (e.g., Cyt II of mB7-1) can be made by preparing overlapping peptides encompassing the amino acid

sequence of the domain and immunizing an animal (e.g., rabbit) with the peptides by standard techniques.

An antibody of the invention can be used to detect novel structural forms of T cell costimulatory molecules. Such an antibody is thus useful for distinguishing between expression by a cell of different forms of T cell costimulatory molecules. For example, a cell which is known to express a costimulatory molecule, such as B7-1, (for example, by the ability of an antibody directed against the extracellular portion of the costimulatory molecule to bind to the cell) can be examined to determine whether the costimulatory molecule includes a novel cytoplasmic domain of the invention. The cell can be reacted with an antibody of the invention by standard immunohistochemical techniques. For example, the antibody can be labeled with a detectable substance and the cells can be permeabilized to allow entry of the antibody into the cell cytoplasm. The antibody is then incubated with the cell and unbound antibody washed away. The presence of the detectable substance associated with the cell is detected as an indication of the binding of the antibody to a novel cytoplasmic domain expressed in the cell. Suitable detectable substances with which to label an antibody include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

C. Kinase Substrates

A novel cytoplasmic domain of the invention which contains a consensus phosphorylation site (i.e., Cyt-II of mB7-1) can be used as a substrate for a protein kinases which phosphorylates the phosphorylation site. Kinase reactions can be performed by standard techniques *in vitro*, e.g., by incubating a polypeptide comprising the cytoplasmic domain (or a T cell costimulatory molecule which includes the novel cytoplasmic domain) with the kinase. The kinase reactions can be performed in the presence of radiolabeled ATP (e.g., ^{32}P - γ -ATP) to radiolabel the novel cytoplasmic domain.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references and published patents and patent applications cited throughout the application are hereby incorporated by reference.

The following methodology was used in the Examples.

Genomic cloning

A mouse 129 lambda genomic library was kindly provided by Drs. Hong Wu and Rudolf Jaenisch of the Whitehead Institute for Biomedical Research, Cambridge, MA. Genomic DNA was prepared from the J1 embryonic stem cell line (derived from the 129/sv mouse strain), partially digested with MboI, sized (17-21 kb), and ligated into the BamHI site of lambda-DASH II arms (Stratagene, La Jolla CA). The library was probed with the coding region of mB7-1 cDNA to yield four clones (λ 4, λ 9, λ 15, and λ 16). These lambda clones were subcloned into Bluescript-pKS II (Stratagene, La Jolla CA) for subsequent restriction mapping.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total cellular RNA was prepared from SWR/J mouse spleen and thymus using RNA-Stat-60 (Tel-Test "B", Inc, Friendswood, Texas). Random hexamer primed reverse transcription (RT) was performed with Superscript-RT (Gibco BRL, Gaithersburg MD) using 1-10 μ g total RNA in a 20 μ l reaction. All PCR reactions were performed in 25 μ l volumes using a manual "hot start", wherein 10X deoxynucleotide triphosphates (dNTPs) were added to the samples at 80 °C. Final reaction conditions were: 60 mM Tris-HCl, pH 8.5, 15 mM $(\text{NH}_4)_2\text{SO}_4$, 2.5 mM MgCl_2 , 200 μ M dNTPs, and 2 μ g/ml each of the specific primers. Cycling conditions for all amplifications were 94° C, 4 minutes prior to 35 cycles of 94° C for 45 seconds, 58° C for 45 seconds, and 72° C for 3 minutes, followed by a final extension at 72° C for 7 minutes. The template for primary PCR was 2 μ l of the RT reaction product and the template for secondary nested PCR was 1 μ l of the primary PCR reaction product.

Oligonucleotides

All oligonucleotides were synthesized on an Applied Biosystems 381A DNA Synthesizer. The oligonucleotides used in this study are listed in Table I and their uses for primary or secondary PCR, as well as sense, also are indicated.

Rapid Amplification of cDNA Ends (RACE) Procedure

Polyadenylated RNA purified by two cycles of oligo-dT selection was obtained from CH1 B lymphoma cells, which express high levels of mB7-1. Primers designed to the most 5' end of the cDNA were employed with the 5' RACE Kit (Gibco BRL, Gaithersburg, MD) according to the manufacturer's instructions. In brief, RNA was reverse transcribed with a gene-specific oligonucleotide, the cDNA purified, and a poly-dCTP tail was added with terminal deoxynucleotide transferase. PCR was performed using a nested primer and an oligonucleotide complimentary to the poly-dCTP tail. PCR bands were cloned, sequenced, and correlated with the genomic sequences.

Oligonucleotide hybridization

Oligonucleotide(s) were 5' end-labeled with polynucleotide kinase and $\gamma^{32}\text{P}$ -ATP. Hybridizations were carried out in 5X SSC and 5% SDS at 55 °C overnight and subsequently washed 3 times for 15 minutes with 2X SSC at 55 °C. Blots were exposed to Kodak XAR-5 film with an intensifying screen at -80 °C.

The oligonucleotides used for the PCR studies in Examples 1-4 are shown in Table I:

Table I. Oligonucleotides used for PCR studies

	<u>Designation</u>	<u>Sequence (5' to 3')</u>	<u>sense</u>	<u>PCR</u>
10	B7.27	CCAACATAACTGAGTCTGGAAA	+	secondary (SEQ ID NO: 43)
	B7.36	CTGGATTCTGACTCACCTTCA	-	secondary (SEQ ID NO: 44)
	B7.37	AGGTAAAGAGTGGTAGAGCCA	-	primary (SEQ ID NO: 45)
	B7.38	AATACCATGTATCCACATGG	-	secondary (SEQ ID NO: 46)
	B7.42	CTGAAGCTATGGCTTGCAATT	+	primary (SEQ ID NO: 47)
15	B7.44	TGGCTTCTCTTTCCTTACCTT	+	secondary (SEQ ID NO: 48)
	B7.48	GCAAATGGTAGATGAGACTGT	-	secondary (SEQ ID NO: 49)
	B7.62	CAACCGAGAAATCTACCAGTAA	-	probe (SEQ ID NO: 50)
	B7.68	GCCGGTAACAAGTCTCTTCA	+	primary (SEQ ID NO: 51)
	B7.71	AAAAGCTCTATAGCATTCTGTC	+	primary (SEQ ID NO: 52)
20	B7.80	ACTGACTTGGACAGTTGTTCA	+	secondary (SEQ ID NO: 53)
	<u>B7.547</u>	<u>TTTGATGGACAACCTTACTA</u>	-	<u>primary (SEQ ID NO: 54)</u>

EXAMPLE 1: Characterization of the mB7-1 genomic locus

Lambda clones containing mB7-1 genomic DNA were isolated using a probe consisting of the coding region of mB7-1. Four representative lambda clones (designated clones $\lambda 4$, $\lambda 9$, $\lambda 15$, and $\lambda 16$) were selected for further analysis. These lambda clones were subcloned and subjected to restriction mapping with HindIII and BamHI. Regions containing exons were further characterized with XbaI and PstI. Fine mapping studies indicate that the mB7-1 locus is comprised of 6 exons arranged in the following 5' to 3' order: 5' UT plus signal peptide domain, Ig-V-like domain, Ig-C-like domain, transmembrane domain, cytoplasmic domain I, and the alternative cytoplasmic domain II, to be discussed below. The 4 lambda clones spanned over 40 kb of the mB7-1 locus, excluding a gap of undetermined size between exon 1 (signal exon) and exon 2 (Ig-V-like exon). The gap between clones $\lambda 15$ (transmembrane domain exon) and $\lambda 16$ (cytoplasmic domain exon) was determined to be less than 100 base pairs by PCR using a sense primer (B7.71) designed to the 3' end of clone $\lambda 15$ and an antisense primer (B7.38) located at the 5' end of clone $\lambda 16$. Clones $\lambda 9$ and $\lambda 15$ overlapped in a region spanning exon 2.

EXAMPLE 2: Identification of mB7-1 exon 6: An alternately spliced exon encoding a novel second cytoplasmic domain

Analysis of mB7-1 cDNAs isolated from an A20 B cell cDNA library showed that one cDNA contained additional sequence not previously described for the mB7-1 cDNA.

5 This sequence was mapped to the mB7-1 locus approximately 7-kb downstream of exon 5. A canonical splice site was present immediately upstream of this sequence and a poly-adenylation site was present downstream. Taken together, these data suggested that this novel sequence represents an additional exon, encoding 46 amino acids, which may be alternatively spliced in place of exon 5. This alternative cytoplasmic domain is notable for
10 two casein kinase II phosphorylation sites (amino acid positions 11-15 (SAKDF) and amino acid positions 28-32 (SLGEA) of SEQ ID NO: 5) (for a description of casein kinase II phosphorylation sites see Pinna (1990) *Biochimica et Biophysica Acta* 1054:267-284) and one protein kinase C phosphorylation site (amino acid positions 11-14 (SAKD) of SEQ ID NO: 5)(for a description of protein kinase C phosphorylation sites see Woodgett et al. (1986)
15 *Biochemistry* 161:177-184; and Kishimoto et al. (1985) *J. Biol. Chem.* 260:12492-12499).

In order to assess whether exon 6 also could be used in an alternative fashion, an antisense primer (B7.48) was designed to the predicted exon 4/6 splice junction such that only the alternatively spliced product would give rise to an amplified product. This primer overhangs the putative exon 4/6 junction by 3 bp at its 3' end. The 3 bp overhang is
20 insufficient to permit direct priming in exon 4 outside the context of an exon 4/6 splice (Figure 1, lane 9, negative control is a cDNA clone containing only mB7-1 CytI). The expected amplified product for the alternately spliced transcript (Figure 1, transcript C) would be 399 bp. Interestingly, this transcript was observed only in thymic, but not splenic RNA.

25 [In Figure 1, lanes 1, 2 and 3 represent nested PCR products from murine splenic RNA using PCR primers B7.27-B7.36, B7.27-B7.38, and B7.27-B7.48, respectively. Lanes 4, 5 and 6 represent nested PCR products from murine thymic RNA using PCR primers B7.27-B7.36, B7.27-B7.38 and B7.27-B7.48, respectively. Lane 7 represents a negative control (no input RNA). Lane 8 represents a positive control (mB7-1 cDNA clone). Lane 9 represents a
30 negative control for B7.27-B7.48 amplification comprised of the mB7-1 cDNA containing cytoplasmic domain I, which does not have the correct exon 4-6 splice junction. Lane M is a 100 bp ladder with the lower bright band equal to 600 bp. Letters A, B and C refer to the transcripts detected and are further illustrated in Figure 1. Note that exon 6 splicing as an alternative cytoplasmic domain is present only in the thymus, but not in the spleen].

35 To further investigate the use of exon 6 in mB7-1 mRNA transcripts, nested RT-PCR spanning exons 3 through 6 was performed using spleen RNA (Figure 1, PCR product A). A PCR product longer than predicted from the use of exon 6 as an alternatively spliced exon also was observed. Subsequent sequence analysis indicated that in this transcript, exons 5 and 6 were spliced in tandem, rather than in an alternative fashion (Figure 1, transcript A),

making use of a previously unrecognized splice donor site downstream of the termination codon in exon 5. Thus, this alternative transcript would not change the encoded protein. Subsequent sequence analysis of a larger than expected product observed from spleen RNA (Figure 1, lane 3) revealed an additional example of the tandem splicing of exon 6 to exon 5 using an alternative noncanonical splice site. Transcripts with tandem splicing of exon 6 to exon 5 were observed in the spleen and the thymus.

Figure 2 is a schematic diagram of the three mB7-1 transcripts (A, B, and C) detected by nested RT-PCR. Exons are depicted in different shades of gray and untranslated sequences are white. Oligonucleotide primers used for the initial RT-PCR and subsequent nested PCR are indicated above their respective locations in the transcripts. Only B7.48 spans an exon-exon junction as indicated. The scale bar above indicates the length in base pairs.

EXAMPLE 3: Identification of additional mB7-1 5' untranslated sequences

Rapid amplification of cDNA ends (RACE) is a PCR-based strategy to determine the 5' end of a transcript. Three distinct rounds of 5' RACE were performed on polyadenylated RNA from CH1 B lymphoma cells, which express high levels of mB7-1 RNA. The resulting sequences extended the 5' UT of the known mB7-1 cDNA by 1505 bp, beyond the transcriptional start site reported by Selvakumar et al. ((1993) *Immunogenetics* 38:292-295). In order to confirm that this long 5' UT sequence was indeed in the mB7-1 mRNA and not generated by PCR amplification of genomic DNA, a nested RT-PCR amplification (B7.68-B7.547 followed by B7.44-B7.80) was performed. This amplification spans exon 2 (primer B7.80) and the novel 5' UT sequences in exon 1 (B7-44), and should yield an 840 bp PCR product. It should be noted that exon 2 is separated from exon 1 by greater than 12 kb in genomic DNA, thus making a genomic DNA-derived PCR product of almost 13kb. The predicted band of 840 bp, indeed, was observed when this nested PCR amplification was performed. To further confirm the nature of the PCR product, hybridization was performed with an oligonucleotide (B7.62) derived from sequences in exon 1 located 5' of the transcriptional start site reported by Selvakumar et al. ((1993) *Immunogenetics* 38:292-295). This probe hybridized to the PCR product. In addition, sequencing of the RACE product revealed that it contained sequences identical to the previously known genomic sequences immediately upstream of the known exon 1 and was contiguous with exon 1. Thus, it did not identify an additional exon.

EXAMPLE 4: Fine mapping of mB7-1 intron-exon boundaries

In order to characterize intron-exon boundaries, oligonucleotide primers were synthesized to mB7-1 cDNA sequences (described in Freeman et al. (1991) *J. Exp. Med.* 174:625-631), as well as to sequences determined from PCR products characterized during amplifications from tissue RNA. Sequences for exons 1 through 5, as well as exon-intron

junctions have been reported previously (Selvakumar et al. (1993) *Immunogenetics* 38:292-295). The coding region of the exon 1 signal peptide domain is 115 bp and is flanked at the 3' end with a canonical splice site. Exons 2 (318 bp), 3 (282 bp), and 4 (114 bp), are separated by 6.0 and 3.8 kb, respectively, and all 3 exons are flanked on both their 5' and 3' ends with canonical splice sites. Exon 5 is located 4 kb downstream of exon 4, and contains a termination codon after the first 97 bp. An additional functional canonical splice site was observed 43 bp downstream of the termination codon in exon 5, since this site was used to generate the transcript outlined in Figure 1 (transcript A). Exon 6 is located 7.2 kb downstream of exon 5 and encodes an open reading frame with a termination codon after 140 bp. Both exons 5 and 6 are followed by polyadenylation sequences, ATTAAG and AATAAG respectively.

EXAMPLE 5: Identification of Additional Novel Cytoplasmic Domains by Exon Trapping

In this example, an exon trapping approach is used to identify a novel exon encoding an alternative cytoplasmic domain for human B7-1. The basic strategy of exon trapping is to create an expression vector encoding a recombinant protein, wherein the encoded protein cannot be functionally expressed unless an appropriate exon, with flanking intron sequences that allow proper mRNA splicing, is cloned into the expression vector. A recombinant expression vector is created comprising transcriptional regulatory sequences (e.g., a strong promoter) linked to nucleic acid encoding the human B7-1 signal peptide exon, IgV-like and IgC-like exons followed by a transmembrane exon with flanking 3' intron donor splice sequences. These splice sequences are immediately followed by translational stop codons in all three frames. A polyadenylation recognition site is not included in the recombinant expression vector. Following the stop codons are restriction enzyme sites which allow genomic DNA fragments to be cloned into the expression vector to create a library of recombinant expression vectors.

As a negative control, the parental recombinant expression vector is transfected into a host cell line which is hB7-1⁻ (e.g, COS cells) and the absence of surface expression of hB7-1 is demonstrated, confirming that the parental expression vector alone is unable to direct stable surface expression of hB7-1 in the absence of a cytoplasmic domain encoding exon. As a positive control, the known hB7-1 cytoplasmic domain with a flanking 5' intron acceptor splice sequence is cloned into a restriction enzyme site downstream of the transmembrane exon such that the transmembrane domain exon can be spliced to the cytoplasmic domain exon. This positive control vector is transfected into a host cell (e.g., COS cells) and the surface expression of hB7-1 on the cells is demonstrated, confirming that the cloning into the vector of a cytoplasmic domain encoding exon with the proper splice sequences produces an hB7-1 molecule that can be stably expressed on the cell surface.

To identify an alternative hB7-1 cytoplasmic domain exon, genomic DNA fragments for the hB7-1 gene are cloned into the parental recombinant expression at the restriction enzyme sites downstream of the transmembrane domain exon. Cloning of genomic fragments into the vector will "trap" DNA fragments which encompass a functional exon preceded by an intron splice acceptor site and followed by a polyadenylation signal, since cloning of such fragments into the vector allows for expression of a functional recombinant protein on the surface of transfected host cells. The diversity of the genomic DNA fragments cloned into the vector directly impacts the variety of sequences "trapped". Were total genomic DNA to be used in such an approach, a variety of exons would be trapped, including cytoplasmic domains from proteins other than T cell costimulatory molecules. However, instead of using total genomic DNA for subcloning into the expression vector, only genomic DNA fragments located in the vicinity of the exon encoding a known cytoplasmic domain of the T cell costimulatory molecule of interest are subcloned into the vector. For example, for human B7-1, genomic DNA clones can be isolated by standard techniques which contain DNA located within several kilobases 5' or 3' of the hB7-1 exon which encodes the known cytoplasmic domain. These fragments are cloned into the parental recombinant expression vector to create a library of expression vectors. The library of expression vectors is then transfected into a host cell (e.g., COS cells) and the transfectants are screened for surface expression of hB7-1. Cell clones which express a functional B7-1 molecule on their surface are identified and affinity purified (e.g., by reacting the cells with a molecule which binds to B7-1, such as an anti-B7-1 monoclonal antibody (e.g., mAb 133 describe in Freedman, A.S. et al. (1987) *J. Immunol.* **137**:3260; and Freeman, G.J. et al. (1989) *J. Immunol.* **143**:2714) or a CTLA4Ig protein (described in Linsley, P.S. et al., (1991) *J. Exp. Med.* **174**:561-569). Cell clones which express a B7-1 molecule on their surface will have incorporated into the expression vector DNA encoding a functional cytoplasmic domain (e.g., an alternative cytoplasmic domain encoded by a different exon than the known cytoplasmic domain). DNA from positive clones encoding the alternative cytoplasmic domain can then be amplified by PCR using a sense primer corresponding to the transmembrane domain and an antisense primer corresponding to vector sequences.

This same approach can be adapted by the skilled artisan to identify alternative cytoplasmic domains for other T cell costimulatory molecules (e.g., B7-2) or to "trap" exons encoding other alternative structural domains of T cell costimulatory molecules.

EXAMPLE 6: Identification of a Novel B7-2 Signal Peptide Domain

cDNA fragments corresponding to the 5' ends of naturally-occurring murine B7-2 mRNA transcripts were prepared by 5' RACE: polyadenylated RNA isolated from murine spleen cells was reverse transcribed with a gene-specific oligonucleotide, the cDNA was isolated, and a poly-dCT tail was added to the 5' end with terminal deoxynucleotide transferase. PCR was performed using a nested primer and an oligonucleotide primer

complementary to the poly-dCTP tail to amplify 5' cDNA fragments of mB7-2 transcripts. The gene-specific oligonucleotide primers used for PCR were as follows:

CAGCTCACTCAGGCTTATGT reverse transcription, - sense (SEQ ID NO: 55)

AAACAGCATCTGAGATCAGCA primary PCR, - sense (SEQ ID NO: 56)

CTGAGATCAGCAAGACTGTC secondary PCR, - sense (SEQ ID NO: 57)

The amplified fragments were subcloned into a plasmid vector and sequenced. Of approximately 100 individual clones examined, ~75 % of the clones had a 5' nucleotide sequence corresponding to that reported for the 5' end of an mB7-2 cDNA (see Freeman, G.J. et al. (1993) *J. Exp. Med.* 178:2185-2192). Approximately 25 % of the clones had a 5' nucleotide sequence shown in SEQ ID NO:14, which encodes a novel signal peptide domain having an amino acid sequence shown in SEQ ID NO:15.

**EXAMPLE 7: Identification of Alternatively Spliced Forms of B7-1
Having a Structural Domain Deleted**

Reverse-transcriptase polymerase chain reaction was used to amplify mB7-1 cDNA fragments derived from murine spleen cell RNA. Oligonucleotide primers used for PCR were as follows:

CTGAAGCTATGGCTTGCAATT primary PCR, + sense (SEQ ID NO: 58)

ACAAGTGTCTTCAGATGTTGAT secondary PCR, + sense (SEQ ID NO: 59)

CTGGATTCTGACTCACCTTCA primary PCR, - sense (SEQ ID NO: 60)

CCAGGTGAAGTCCTCTGACA secondary PCR, - sense (SEQ ID NO: 61)

A cDNA fragment was detected which comprises a nucleotide sequence (SEQ ID NO:8) encoding a murine B7-1 molecule in which the signal peptide domain was spliced directly to the IgC-like domain (i.e., the IgV-like domain was deleted). The amino acid sequence of mB7-1 encoded by this cDNA is shown in SEQ ID NO:9.

Another cDNA fragment was detected with comprises a nucleotide sequence (SEQ ID NO: 62) encoding a murine B7-1 molecule in which the IgV-like domain was spliced directly to the transmembrane domain (i.e., the IgC-like domain was deleted). The amino acid sequence encoded by this cDNA is shown in SEQ ID NO: 63). This protein is referred to herein as an IgV-like isoform of mB7-1. To examine the functional activity of the IgV-like

isoform of mB7-1, its cDNA was cloned into an expression vector, pBK-CMV, in which transcription of the cDNA is placed under the control of the CMV promoter. The expression vector was cotransfected into Chinese Hamster Ovary (CHO) cells, along with a puromycin resistance gene, and drug resistant clones were selected. The resultant clones expressing the IgV-like isoform of mB7-1 on their surface are referred to herein as CHO-sV clones.

Expression of the IgV-like isoform of mB7-1 on the surface of the CHO-sV cells was confirmed by FACS analysis using either murine CTLA4Ig, murine CD28Ig or anti-B7-1 antibody as the primary staining reagent. Each of these reagents stained the CHO-sV cells. Positive staining of CHO-sV with both mCTLA4Ig and mCD28Ig indicate that the IgV-like isoform of mB7-1 is capable of interacting with both CTLA4 and CD28. In contrast to the results with mouse CTLA4Ig, human CTLA4Ig failed to stain the CHO-sV cells, although this reagent was able to stain CHO cells expressing the full-length mouse B7-1 molecule (CHO-B7-1 cells). These data implicate the IgC domain of mB7-1 in the binding to human CTLA4Ig, whereas the IgC domain of mB7-1 is not required for binding to mouse CTLA4Ig. These results suggest species differences in the binding parameters for human and murine CTLA4.

The ability of the IgV-like isoform of mB7-1 on CHO-sV cells to deliver a costimulatory signal to T cells was tested in standard T cell proliferation and interleukin-2 (IL-2) production assays. T cells that received a primary activation signal were stimulated to produce IL-2 when incubated with either CHO-B7-1 cells or CHO-sV cells but not when incubated with untransfected CHO cells. The results of this experiment is illustrated graphically in Figure 3, in which IL-2 production by T cells is expressed as a function of the number of CHO cells used to costimulate the T cells. The data demonstrate that CHO-sV cells can trigger a costimulatory signal in T cells, although the level of IL-2 production by cells stimulated with CHO-sV was approximately 25-50% of the level of IL-2 production by cells stimulated with CHO-B7-1. Similar results were observed when T cell proliferation was assayed as an indicator of T cell costimulation.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

(A) NAME: BRIGHAM AND WOMEN'S HOSPITAL

(B) STREET: 75 FRANCIS STREET

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(F) POSTAL CODE (ZIP): 02115

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(F) POSTAL CODE (ZIP): 02115

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(ii) TITLE OF INVENTION: Novel Forms of T Cell Costimulatory Molecules
and Uses Therefor

(iii) NUMBER OF SEQUENCES: 65

25

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30

(E) COUNTRY: USA

(F) ZIP: 02109-1875

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

35

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: ASCII Text

(vi) CURRENT APPLICATION DATA:

40

(A) APPLICATION NUMBER:

(B) FILING DATE:

(vi) PRIOR APPLICATION DATA:

45

(A) APPLICATION NUMBER: US 08/205,697

(B) FILING DATE: 02-Mar-1994

(viii) ATTORNEY/AGENT INFORMATION:

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50

(C) REFERENCE/DOCKET NUMBER: BWI-120CPPC

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55

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1888 base pairs

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

10 (A) NAME/KEY: CDS
 (B) LOCATION: 249..1208

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15	GAGTTTTATA CCTCAATAGA CTCTTACTAG TTTCTCTTTT TCAGGTTGTG AAACCTCAACC	60
	TTCAAAGACA CTCTGTTCCA TTTCTGTGGA CTAATAGGAT CATCTTTAGC ATCTGCCGGG	120
20	TGGATGCCAT CCAGGCTTCT TTTTCTACAT CTCTGTTTCT CGATTTTGTG GAGCCTAGGA	180
	GGTGCCTAAG CTCCATTGGC TCTAGATTCC TGGCTTTCCC CATCATGTTC TCCAAAGCAT	240
	CTGAAGCT ATG GCT TGC AAT TGT CAG TTG ATG CAG GAT ACA CCA CTC CTC	290
25	Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu	
	1 5 10	
	AAG TTT CCA TGT CCA AGG CTC AAT CTT CTC TTT GTG CTG CTG ATT CGT	338
	Lys Phe Pro Cys Pro Arg Leu Asn Leu Leu Phe Val Leu Leu Ile Arg	
30	15 20 25 30	
	CTT TCA CAA GTG TCT TCA GAT GTT GAT GAA CAA CTG TCC AAG TCA GTG	386
	Leu Ser Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val	
	35 40 45	
35	AAA GAT AAG GTA TTG CTG CCT TGC CGT TAC AAC TCT CCT CAT GAA GAT	434
	Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp	
	50 55 60	
40	GAG TCT GAA GAC CGA ATC TAC TGG CAA AAA CAT GAC AAA GTG GTG CTG	482
	Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu	
	65 70 75	
	TCT GTC ATT GCT GGG AAA CTA AAA GTG TGG CCC GAG TAT AAG AAC CGG	530
45	Ser Val Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg	
	80 85 90	
	ACT TTA TAT GAC AAC ACT ACC TAC TCT CTT ATC ATC CTG GGC CTG GTC	578
	Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val	
50	95 100 105 110	
	CTT TCA GAC CGG GGC ACA TAC AGC TGT GTC GTT CAA AAG AAG GAA AGA	626
	Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg	
	115 120 125	
55	GGA ACG TAT GAA GTT AAA CAC TTG GCT TTA GTA AAG TTG TCC ATC AAA	674
	Gly Thr Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys	
	130 135 140	

	GCT GAC TTC TCT ACC CCC AAC ATA ACT GAG TCT GGA AAC CCA TCT GCA	722
	Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala	
	145 150 155	
5	GAC ACT AAA AGG ATT ACC TGC TTT GCT TCC GGG GGT TTC CCA AAG CCT	770
	Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro	
	160 165 170	
10	CGC TTC TCT TGG TTG GAA AAT GGA AGA GAA TTA CCT GGC ATC AAT ACG	818
	Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr	
	175 180 185 190	
15	ACA ATT TCC CAG GAT CCT GAA TCT GAA TTG TAC ACC ATT AGT AGC CAA	866
	Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln	
	195 200 205	
20	CTA GAT TTC AAT ACG ACT CGC AAC CAC ACC ATT AAG TGT CTC ATT AAA	914
	Leu Asp Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys	
	210 215 220	
25	TAT GGA GAT GCT CAC GTG TCA GAG GAC TTC ACC TGG GAA AAA CCC CCA	962
	Tyr Gly Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro	
	225 230 235	
30	GAA GAC CCT CCT GAT AGC AAG AAC ACA CTT GTG CTC TTT GGG GCA GGA	1010
	Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly	
	240 245 250	
35	TTC GGC GCA GTA ATA ACA GTC GTC GTC ATC GTT GTC ATC ATC AAA TGC	1058
	Phe Gly Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys	
	255 260 265 270	
40	TTC TGT AAG CAC GGT CTC ATC TAC CAT TTG CAA CTG ACC TCT TCT GCA	1106
	Phe Cys Lys His Gly Leu Ile Tyr His Leu Gln Leu Thr Ser Ser Ala	
	275 280 285	
45	AAG GAC TTC AGA AAC CTA GCA CTA CCC TGG CTC TGC AAA CAC GGT TCT	1154
	Lys Asp Phe Arg Asn Leu Ala Leu Pro Trp Leu Cys Lys His Gly Ser	
	290 295 300	
50	CTA GGT GAA GCC TCT GCA GTG ATT TGC AGA AGT ACT CAG ACG AAT GAA	1202
	Leu Gly Glu Ala Ser Ala Val Ile Cys Arg Ser Thr Gln Thr Asn Glu	
	305 310 315	
55	CCA CAG TAGTTCTGCT GTTCTGAGG ACGTAGTTTA GAGACTGAAT TCTTTGAAA	1258
	Pro Gln	
	320	
60	GGACATAGGG ACAGTTTGCA CATTTGCTTG CACATCACAC ACACACACAC ACACACACAC	1318
65	ACACACACAC ACACACACAC ACACACACAC ACACACACAC TCTCTCTCTC TCTCTCTCTC	1378
70	GATACCTTAG GATAGGGTTC TACCCTGTTG CTCAGTGACA AAGAATCACT CTGTGGCGGA	1438
75	GGCAGGCTTC AAGCTTGCAG CAATCCTCCT GCACCAGTTT CCTGAGTGCC AGACTTCCAG	1498
80	GTGTAAGCTA TGGCACTTAG CAGAACACTA GCTGAATCAA TGAAGACACT GAGGTTCCAA	1558
85	GAGGGAACCT GAATTATGAA GGTGAGTCAG AATCCAGATT TCCTGGCTCT ACCACTCTTA	1618

ACCTGTATCT GTTAGACCCC AAGCTCTGAG CTCATAGACA AGCTAATTTA AAATGCTTTT 1678
 TAATAAGCAG AAGGCTCAGT TAGTACGGGG TTCAGGATAC TGCTTACTGG CAATATTTGA 1738
 5 CTAGCCTCTA TTTTGTTTGT TTTTAAAGG CCTACTGACT GTAGTGTAAT TTGTAGGAAA 1798
 CATGTTGCTA TGTATACCCA TTTGAGGGTA ATAAAAATGT TGGTAATTTT CAGCCAGCAC 1858
 10 TTTCCAGGTA TTTCCCTTTT TATCCTTCAT 1888

(2) INFORMATION FOR SEQ ID NO:2:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 320 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25 Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu Lys Phe
 1 5 10 15
 Pro Cys Pro Arg Leu Asn Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
 20 25 30
 30 Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp
 35 40 45
 Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser
 50 55 60
 35 Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu Ser Val
 65 70 75 80
 40 Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu
 85 90 95
 Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser
 100 105 110
 45 Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr
 115 120 125
 Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp
 130 135 140
 50 Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr
 145 150 155 160
 55 Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe
 165 170 175
 Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile
 180 185 190

Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp
 195 200 205

5 Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly
 210 215 220

Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp
 225 230 235 240

10 Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly Phe Gly
 245 250 255

Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys Phe Cys
 260 265 270

15 Lys His Gly Leu Ile Tyr His Leu Gln Leu Thr Ser Ser Ala Lys Asp
 275 280 285

20 Phe Arg Asn Leu Ala Leu Pro Trp Leu Cys Lys His Gly Ser Leu Gly
 290 295 300

Glu Ala Ser Ala Val Ile Cys Arg Ser Thr Gln Thr Asn Glu Pro Gln
 305 310 315 320

25 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 2516 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35 (ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 249..1166

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

45 GAGTTTATA CCTCAATAGA CTCTTACTAG TTTCTCTTTT TCAGGTTGTG AAACCTCAACC 60
 TTCAAAGACA CTCTGTTCCA TTTCTGTGGA CTAATAGGAT CATCTTTAGC ATCTGCCGGG 120
 TGGATGCCAT CCAGGCTTCT TTTTCTACAT CTCTGTTTCT CGATTTTTGT GAGCCTAGGA 180
 50 GGTGCCTAAG CTCCATTGGC TCTAGATTCC TGGCTTTCCC CATCATGTTC TCAAAGCAT 240
 CTGAAGCT ATG GCT TGC AAT TGT CAG TTG ATG CAG GAT ACA CCA CTC CTC 290
 Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu
 1 5 10

55 AAG TTT CCA TGT CCA AGG CTC AAT CTT CTC TTT GTG CTG CTG AAT CGT 338
 Lys Phe Pro Cys Pro Arg Leu Asn Leu Leu Phe Val Leu Leu Asn Arg
 15 20 25 30

	CTT TCA CAA GTG TCT TCA GAT GTT GAT GAA CAA CTG TCC AAG TCA GTG	386
	Leu Ser Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val	
	35 40 45	
5	AAA GAT AAG GTA TTG CTG CCT TGC CGT TAC AAC TCT CCT CAT GAA GAT	434
	Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp	
	50 55 60	
10	GAG TCT GAA GAC CGA ATC TAC TGG CAA AAA CAT GAC AAA GTG GTG CTG	482
	Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu	
	65 70 75	
15	TCT GTC ATT GCT GGG AAA CTA AAA GTG TGG CCC GAG TAT AAG AAC CGG	530
	Ser Val Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg	
	80 85 90	
20	ACT TTA TAT GAC AAC ACT ACC TAC TCT CTT ATC ATC CTG GGC CTG GTC	578
	Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val	
	95 100 105 110	
	CTT TCA GAC CGG GGC ACA TAC AGC TGT GTC GTT CAA AAG AAG GAA AGA	626
	Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg	
	115 120 125	
25	GGA ACG TAT GAA GTT AAA CAC TTG GCT TTA GTA AAG TTG TCC ATC AAA	674
	Gly Thr Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys	
	130 135 140	
30	GCT GAC TTC TCT ACC CCC AAC ATA ACT GAG TCT GGA AAC CCA TCT GCA	722
	Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala	
	145 150 155	
35	GAC ACT AAA AGG ATT ACC TGC TTT GCT TCC GGG GGT TTC CCA AAG CCT	770
	Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro	
	160 165 170	
40	CGC TTC TCT TGG TTG GAA AAT GGA AGA GAA TTA CCT GGC ATC AAT ACG	818
	Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr	
	175 180 185 190	
	ACA ATT TCC CAG GAT CCT GAA TCT GAA TTG TAC ACC ATT AGT AGC CAA	866
	Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln	
	195 200 205	
45	CTA GAT TTC AAT ACG ACT CGC AAC CAC ACC ATT AAG TGT CTC ATT AAA	914
	Leu Asp Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys	
	210 215 220	
50	TAT GGA GAT GCT CAC GTG TCA GAG GAC TTC ACC TGG GAA AAA CCC CCA	962
	Tyr Gly Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro	
	225 230 235	
55	GAA GAC CCT CCT GAT AGC AAG AAC ACA CTT GTG CTC TTT GGG GCA GGA	1010
	Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly	
	240 245 250	
	TTC GGC GCA GTA ATA ACA GTC GTC GTC ATC GTT GTC ATC ATC AAA TGC	1058
	Phe Gly Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys	
	255 260 265 270	

	TTC TGT AAG CAC AGA AGC TGT TTC AGA AGA AAT GAG GCA AGC AGA GAA	1106
	Phe Cys Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu	
	275 280 285	
5	ACA AAC AAC AGC CTT ACC TTC GGG CCT GAA GAA GCA TTA GCT GAA CAG	1154
	Thr Asn Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln	
	290 295 300	
10	ACC GTC TTC CTT TAGTTCTTCT CTGTCCATGT GGGATACATG GTATTATGTG	1206
	Thr Val Phe Leu	
	305	
15	GCTCATGAGG TACAATCTTT CTTTCAGCAC CGTGCTAGCT GATCTTTCGG ACAACTTGAC	1266
	ACAAGATAGA GTTAACTGGG AAGAGAAAGC CTTGAATGAG GATTTCTTTC CATCAGGAAG	1326
	CTACGGGCAA GTTTGCTGGG CCTTTGATTG CTTGATGACT GAAGTGGAAA GGCTGAGCCC	1386
20	ACTGTGGGTG GTGCTAGCCC TGGGCAGGGG CAGGTGACCC TGGGTGGTAT AAGAAAAAGA	1446
	GCTGTCACTA AAAGGAGAGG TGCCTAGTCT TACTGCAACT TGATATGTCA TGTTTGTTG	1506
	GTGTCTGTGG GAGGCCTGCC CTTTTCTGAA GAGAAGTGGT GGGAGAGTGG ATGGGGTGGG	1566
25	GGCAGAGGAA AAGTGGGGGA GAGGGCCTGG GAGGAGAGGA GGGAGGGGGA CGGGGTGGGG	1626
	GTGGGGAAAA CTATGGTTGG GATGTAAAAA CGGATAATAA TATAAATATT AAATAAAAAG	1686
30	AGAGTATTGA GCGGTCTCAT CTACCATTTC CAACTGACCT CTTCTGCAA GGAATTCAGA	1746
	AACCTAGCAC TACCCTGGCT CTGCAAACAC GGTTCCTCTAG GTGAAGCCTC TGCAGTGATT	1806
	TGCAGAAGTA CTCAGACGAA TGAACCACAG TAGTTCTGCT GTTTCTGAGG ACGTAGTTTA	1866
35	GAGACTGAAT TCTTTGGAAA GGACATAGGG ACAGTTTGCA CATTTGCTTG CACATCACAC	1926
	ACACACACAC ACACACACAC ACACACACAC ACACACACAC ACACACACAC ACACACACAC	1986
40	TCTCTCTCTC TCTCTCTCTC GATACCTTAG GATAGGGTTC TACCCTGTTG CTCAGTGACA	2046
	AAGAATCACT CTGTGGCGGA GGCAGGCTTC AAGCTTGCAG CAATCCTCCT GCACCAGTTT	2106
	CCTGAGTGCC AGACTTCCAG GTGTAAGCTA TGGCACTTAG CAGAACACTA GCTGAATCAA	2166
45	TGAAGACACT GAGGTTCCAA GAGGGAACCT GAATTATGAA GGTGAGTCAG AATCCAGATT	2226
	TCCTGGCTCT ACCACTCTTA ACCTGTATCT GTTAGACCCC AAGCTCTGAG CTCATAGACA	2286
50	AGCTAATTTA AAATGCTTTT TAATAAGCAG AAGGCTCAGT TAGTACGGGG TTCAGGATAC	2346
	TGCTTACTGG CAATATTTGA CTAGCCTCTA TTTTGTTTGT TTTTAAAGG CCTACTGACT	2406
	GTAGTGTAAT TTGTAGGAAA CATGTTGCTA TGTATACCCA TTTGAGGGTA ATAAAAATGT	2466
55	TGGTAATTTT CAGCCAGCAC TTTCCAGGTA TTTCCCTTTT TATCCTTCAT	2516

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 818 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..138

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGT CTC ATC TAC CAT TTG CAA CTG ACC TCT TCT GCA AAG GAC TTC AGA	48
Gly Leu Ile Tyr His Leu Gln Leu Thr Ser Ser Ala Lys Asp Phe Arg	
1 5 10 15	
AAC CTA GCA CTA CCC TGG CTC TGC AAA CAC GGT TCT CTA GGT GAA GCC	96
Asn Leu Ala Leu Pro Trp Leu Cys Lys His Gly Ser Leu Gly Glu Ala	
20 25 30	
TCT GCA GTG ATT TGC AGA AGT ACT CAG ACG AAT GAA CCA CAG	138
Ser Ala Val Ile Cys Arg Ser Thr Gln Thr Asn Glu Pro Gln	
35 40 45	
TAGTTCTGCT GTTTCTGAGG ACGTAGTTTA GAGACTGAAT TCTTTGGAAA GGACATAGGG	198
ACAGTTTGCA CATTTGCTTG CACATCACAC ACACACACAC ACACACACAC ACACACACAC	258
ACACACACAC ACACACACAC ACACACACAC TCTCTCTCTC TCTCTCTCTC GATACCTTAG	318
GATAGGGTTC TACCCTGTTG CTCAGTGACA AAGAATCACT CTGTGGCGGA GGCAGGCTTC	378
AAGCTTGCAG CAATCCTCCT GCACCAGTTT CCTGAGTGCC AGACTTCCAG GTGTAAGCTA	438
TGGCACTTAG CAGAACTA GCTGAATCAA TGAAGACACT GAGGTTCCAA GAGGGAACCT	498
GAATTATGAA GGTGAGTCAG AATCCAGATT TCCTGGCTCT ACCACTCTTA ACCTGTATCT	558
GTTAGACCCC AAGCTCTGAG CTCATAGACA AGCTAATTTA AAATGCTTTT TAATAAGCAG	618
AAGGCTCAGT TAGTACGGGG TTCAGGATAC TGCTTACTGG CAATATTTGA CTAGCCTCTA	678
TTTTGTTTGT TTTTAAAGG CCTACTGACT GTAGTGTAAT TTGTAGGAAA CATGTTGCTA	738
TGTATACCCA TTTGAGGGTA ATAAAAATGT TGGTAATTTT CAGCCAGCAC TTTCCAGGTA	798
TTCCCTTTT TATCCTTCAT	818

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Leu Ile Tyr His Leu Gln Leu Thr Ser Ser Ala Lys Asp Phe Arg
1 5 10 15
Asn Leu Ala Leu Pro Trp Leu Cys Lys His Gly Ser Leu Gly Glu Ala
20 25 30
Ser Ala Val Ile Cys Arg Ser Thr Gln Thr Asn Glu Pro Gln
35 40 45

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 1753 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

30 GTTTTAGTAA CCAGAGGCCG CAAGAAGAGA TCACTTGTAT ATACACGGGC CCCATCTTTT 60
GCTTTTAAAG ACAAAGAAA AAGAATCTTC TTCAACAAGT AAGTAAATGC ATTTACTATT 120
TATCATGCTA TGGGACACCT TAGTAGAACA CGCTATCTCC AGCCTTATCA TATGCATATT 180
35 TTGTTGTTGT TGTGTTGTT GTTGTTAAAG ACAGGGTCTC ATATATGCCA GGCTGGTCCC 240
AAACTTTCAG TGTAACCCAA GATAATCTGG AACTCCCGAC TCCTCTGCTC CCACCTCTCC 300
AGTGCAGGAC ACTGTTTATA CCGTGCTGGG GAATTGAACT CAGAGCACCC TGCATGTCAG 360
40 CTAAGCATTC TACCGACCAA GTCCCATGCC CAGTCCCTAA CTCCCCAACT TCACTGCTTT 420
TTAAACATAC ATACAATCAT AACTTGCCCT CAGAGCAGTC TCCTGGGGTC TCTTATTCTC 480
45 AAGGCTGCGG CATTCCAACA CTGTTAGAAA AACACCATCA GGATTCTTTT GTGTTTCCTA 540
GATGCAAACA TTTTGTAGG GCGAAGTTGA GGTTTTTCTA ATCAAGAAA TGCCGGTAAC 600
AAGTCTCTTC AAGCTAACTG GTTGGCTAAG GGGTATCTCT CCAAAGAAG AGATCCACAT 660
50 GTCAGGCCAG TTGTAGGCAT GATGTCAGGT CTCCCTCCCT TTCTTTCTTT CTTTCTTTTT 720
TTCTTTCTTT CTTTTTTTCT TTCTTTCTTA CTTTCTTACT TTCTTTCTTT TCTGTTTTTT 780
55 GGTTTTTCGA GACAGGGTTT CTTTGTATAG CCCTGGCTGT CCTGGAAGTC GCTCTGTAGA 840
CCAGGCTGGC CTCGAACTCA GAAATCTGCC TCTGCCTTTA CCTCCTGAGT GCTGGGAATT 900
AAAGGTGTGC ACCACCATGC CCGGCTGGGA TGTCATTCGT TTTCATTTCT CAATTTTGAT 960

ACTTTATGGA AGAAAAAAGA AAAGATAGAC AAGCCTCTTC ATGTAATACC CCATAGTCTC 1020
AATAAGTGGT GTTCGTAACG TGGCTTCTCT TTCCTTACCT TTTACTGGTA GATTTCTCGG 1080
5 TTGATTGATG TCCCTGTAGG ACTTACTGGG TTTAAGATTC TTGGTTTCCT GTTTTAAGAT 1140
ATAAAGAAAC CATTTCTTAA CTAAAACACT GCCTTGACAA AATATACTTT TGGCAGTCAC 1200
10 TCTGTGTCCA GAATGGAATT TAAGCTTTCA TGGCCTAGCT GCTAGTGAAG GTTCTTTGCT 1260
TTTTTTTGGC TGTTGTATGT GAAATGGGGT TGGGTGGGAA CCACCTCACT GTGTTCTAGT 1320
GTTAGTCACC CCACCCCCGC AAGCAGAATC CTTTTACCCA GCTTTTTCAC CCAGCTGTGC 1380
15 TCACCCGGTG CTCAGAACAG GCCTGGACAA GTCACCTCCC CTAGAGTTCT GGGGACCTTT 1440
GAGTTGCCCT CATGGCCACA CCCTGATTCA GAACTCTCAC TCTGTCGTAA GATAGAGCTA 1500
20 CTGGGGAGTT TTATACCTCA ATAGACTCTT ACTAGTTTCT CTTTTTCAGG TTGTGAAACT 1560
CAACCTTCAA AGACACTCTG TTCCATTTCT GTGGACTAAT AGGATCATCT TTAGCATCTG 1620
CCGGGTGGAT GCCATCCAGG CTTCTTTTTC TACATCTCTG TTTCTCGATT TTTGTGAGCC 1680
25 TAGGAGGTGC CTAAGCTCCA TTGGCTCTAG ATTCCTGGCT TTCCCCATCA TGTTCTCCAA 1740
AGCATCTGAA GCT 1753

30 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 158 base pairs

(B) TYPE: nucleic acid

35 (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGTCCAGGCA GAGCTAGTGG CTGCCCCTAG CGCTTCCTCT TCTTTGATAC CCCAAAGTCT 60

GAGTTTATTA CACATCCTTG GTGACCAAAT CACATGGGAG CTTCTCCGA GGTCTTAGTA 120

45 AAGGGAAGTT GGAAAGGGGA AATTCCTGCC CCCCTGCC 158

(2) INFORMATION FOR SEQ ID NO:8:

50 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1398 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 249..848

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

5 GAGTTTTATA CCTCAATAGA CTCTTACTAG TTTCTCTTTT TCAGGTTGTG AAACTCAACC 60

TTCAAAGACA CTCTGTTCCA TTTCTGTGGA CTAATAGGAT CATCTTTAGC ATCTGCCGGG 120

10 TGGATGCCAT CCAGGCTTCT TTTTCTACAT CTCTGTTTCT CGATTTTGTG GAGCCTAGGA 180

GGTGCCTAAG CTCCATTGGC TCTAGATTCC TGGCTTTCCC CATCATGTTC TCCAAAGCAT 240

15 CTGAAGCT ATG GCT TGC AAT TGT CAG TTG ATG CAG GAT ACA CCA CTC CTC 290

Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu

1 5 10

AAG TTT CCA TGT CCA AGG CTC AAT CTT CTC TTT GTG CTG CTG ATT CGT 338

Lys Phe Pro Cys Pro Arg Leu Asn Leu Leu Phe Val Leu Leu Ile Arg

20 15 20 25 30

CTT TCA CAA GTG TCT TCA GCT GAC TTC TCT ACC CCC AAC ATA ACT GAG 386

Leu Ser Gln Val Ser Ser Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu

35 40 45

25 TCT GGA AAC CCA TCT GCA GAC ACT AAA AGG ATT ACC TGC TTT GCT TCC 434

Ser Gly Asn Pro Ser Ala Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser

50 55 60

30 GGG GGT TTC CCA AAG CCT CGC TTC TCT TGG TGG GAA AAT GGA AGA GAA 482

Gly Gly Phe Pro Lys Pro Arg Phe Ser Trp Trp Glu Asn Gly Arg Glu

65 70 75

35 TTA CCT GGC ATC AAT ACG ACA ATT TCC CAG GAT CCT GAA TCT GAA TTG 530

Leu Pro Gly Ile Asn Thr Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu

80 85 90

40 TAC ACC ATT AGT AGC CAA CTA GAT TTC AAT ACG ACT CGC AAC CAC ACC 578

Tyr Thr Ile Ser Ser Gln Leu Asp Phe Asn Thr Thr Arg Asn His Thr

95 100 105 110

ATT AAG TGT CTC ATT AAA TAT GGA GAT GCT CAC GTG TCA GAG GAC TTC 626

Ile Lys Cys Leu Ile Lys Tyr Gly Asp Ala His Val Ser Glu Asp Phe

115 120 125

45 ACC TGG GAA AAA CCC CCA GAA GAC CCT CCT GAT AGC AAG AAC ACA CTT 674

Thr Trp Glu Lys Pro Pro Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu

130 135 140

50 GTG CTC TTT GGG GCA GGA TTC GGC GCA GTA ATA ACA GTC GTC GTC ATC 722

Val Leu Phe Gly Ala Gly Phe Gly Ala Val Ile Thr Val Val Val Ile

145 150 155

55 GTT GTC ATC ATC AAA TGC TTC TGT AAG CAC AGA AGC TGT TTC AGA AGA 770

Val Val Ile Ile Lys Cys Phe Cys Lys His Arg Ser Cys Phe Arg Arg

160 165 170

AAT GAG GCA AGC AGA GAA ACA AAC AAC AGC CTT ACC TTC GGG CCT GAA 818
 Asn Glu Ala Ser Arg Glu Thr Asn Asn Ser Leu Thr Phe Gly Pro Glu
 175 180 185 190

5 GAA GCA TTA GCT GAA CAG ACC GTC TTC CTT TAGTTCTTCT CTGTCCATGT 868
 Glu Ala Leu Ala Glu Gln Thr Val Phe Leu
 195 200

10 GGGATACATG GTATTATGTG GCTCATGAGG TACAATCTTT CTTTCAGCAC CGTGCTAGCT 928
 GATCTTTTCGG ACAACTTGAC ACAAGATAGA GTTAACTGGG AAGAGAAAGC CTTGAATGAG 988
 GATTTCTTTC CATCAGGAAG CTACGGGCAA GTTTGCTGGG CCTTTGATTG CTTGATGACT 1048

15 GAAGTGGAAG GGCTGAGCCC ACTGTGGGTG GTGCTAGCCC TGGGCAGGGG CAGGTGACCC 1108
 TGGGTGGTAT AAGAAAAAGA GCTGTCACTA AAAGGAGAGG TGCCTAGTCT TACTGCAACT 1168
 20 TGATATGTCA TGTTTGGTTG GTGTCTGTGG GAGGCCTGCC CTTTTCTGAA GAGAAGTGGT 1228
 GGGAGAGTGG ATGGGGTGGG GGCAGAGGAA AAGTGGGGGA GAGGGCCTGG GAGGAGAGGA 1288
 GGGAGGGGGA CGGGGTGGGG GTGGGGAAAA CTATGGTTGG GATGTAAAAA CGGATAATAA 1348

25 TATAAATATT AAATAAAAAG AGAGTATTGA GCAAAAAAAA AAAAAAAAAA 1398

(2) INFORMATION FOR SEQ ID NO:9:

- 30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 200 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- 35 (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

40 Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu Lys Phe
 1 5 10 15

Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
 20 25 30

45 Gln Val Ser Ser Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly
 35 40 45

Asn Pro Ser Ala Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly
 50 55 60

Phe Pro Lys Pro Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro
 65 70 75 80

55 Gly Ile Asn Thr Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr
 85 90 95

Ile Ser Ser Gln Leu Asp Phe Asn Thr Thr Arg Asn His Thr Ile Lys
 100 105 110

Cys Leu Ile Lys Tyr Gly Asp Ala His Val Ser Glu Asp Phe Thr Trp
 115 120 125

5 Glu Lys Pro Pro Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu Val Leu
 130 135 140

Phe Gly Ala Gly Phe Gly Ala Val Ile Thr Val Val Val Ile Val Val
 145 150 155 160

10 Ile Ile Lys Cys Phe Cys Lys His Arg Ser Cys Phe Arg Arg Asn Glu
 165 170 175

Ala Ser Arg Glu Thr Asn Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala
 180 185 190

15 Leu Ala Glu Gln Thr Val Phe Leu
 195 200

(2) INFORMATION FOR SEQ ID NO:10:

20

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1570 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 249..890

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GAGTTTTATA CCTCAATAGA CTCTTACTAG TTTCTCTTTT TCAGGTTGTG AAACCTCAACC 60

40

TTCAAAGACA CTCTGTTCCA TTTCTGTGGA CTAATAGGAT CATCTTTAGC ATCTGCCGGG 120

TGGATGCCAT CCAGGCTTCT TTTTCTACAT CTCTGTTTCT CGATTTTGTG GAGCCTAGGA 180

GGTGCCTAAG CTCCATTGGC TCTAGATTCC TGGCTTTCCC CATCATGTTC TCCAAAGCAT 240

45

CTGAAGCT ATG GCT TGC AAT TGT CAG TTG ATG CAG GAT ACA CCA CTC CTC 290
 Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu
 1 5 10

50

AAG TTT CCA TGT CCA AGG CTC AAT CTT CTC TTT GTG CTG CTG ATT CGT 338
 Lys Phe Pro Cys Pro Arg Leu Asn Leu Leu Phe Val Leu Leu Ile Arg
 15 20 25 30

55

CTT TCA CAA GTG TCT TCA GCT GAC TTC TCT ACC CCC AAC ATA ACT GAG 386
 Leu Ser Gln Val Ser Ser Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu
 35 40 45

TCT GGA AAC CCA TCT GCA GAC ACT AAA AGG ATT ACC TGC TTT GCT TCC 434
 Ser Gly Asn Pro Ser Ala Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser
 50 55 60

	GGG GGT TTC CCA AAG CCT CGC TTC TCT TGG TTG GAA AAT GGA AGA GAA	482
	Gly Gly Phe Pro Lys Pro Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu	
	65 70 75	
5	TTA CCT GGC ATC AAT ACG ACA ATT TCC CAG GAT CCT GAA TCT GAA TTG	530
	Leu Pro Gly Ile Asn Thr Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu	
	80 85 90	
10	TAC ACC ATT AGT AGC CAA CTA GAT TTC AAT ACG ACT CGC AAC CAC ACC	578
	Tyr Thr Ile Ser Ser Gln Leu Asp Phe Asn Thr Thr Arg Asn His Thr	
	95 100 105 110	
15	ATT AAG TGT CTC ATT AAA TAT GGA GAT GCT CAC GTG TCA GAG GAC TTC	626
	Ile Lys Cys Leu Ile Lys Tyr Gly Asp Ala His Val Ser Glu Asp Phe	
	115 120 125	
20	ACC TGG GAA AAA CCC CCA GAA GAC CCT CCT GAT AGC AAG AAC ACA CTT	674
	Thr Trp Glu Lys Pro Pro Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu	
	130 135 140	
25	GTG CTC TTT GGG GCA GGA TTC GGC GCA GTA ATA ACA GTC GTC GTC ATC	722
	Val Leu Phe Gly Ala Gly Phe Gly Ala Val Ile Thr Val Val Val Ile	
	145 150 155	
30	GTT GTC ATC ATC AAA TGC TTC TGT AAG CAC GGT CTC ATC TAC CAT TTG	770
	Val Val Ile Ile Lys Cys Phe Cys Lys His Gly Leu Ile Tyr His Leu	
	160 165 170	
35	CAA CTG ACC TCT TCT GCA AAG GAC TTC AGA AAC CTA GCA CTA CCC TGG	818
	Gln Leu Thr Ser Ser Ala Lys Asp Phe Arg Asn Leu Ala Leu Pro Trp	
	175 180 185 190	
40	CTC TGC AAA CAC GGT TCT CTA GGT GAA GCC TCT GCA GTG ATT TGC AGA	866
	Leu Cys Lys His Gly Ser Leu Gly Glu Ala Ser Ala Val Ile Cys Arg	
	195 200 205	
45	AGT ACT CAG ACG AAT GAA CCA CAG TAGTTCTGCT GTTCTGAGG ACGTAGTTTA	920
	Ser Thr Gln Thr Asn Glu Pro Gln	
	210	
50	GAGACTGAAT TCTTTGGAAA GGACATAGGG ACAGTTTGCA CATTTGCTTG CACATCACAC	980
	ACACACACAC ACACACACAC ACACACACAC ACACACACAC ACACACACAC ACACACACAC	1040
	TCTCTCTCTC TCTCTCTCTC GATACCTTAG GATAGGGTTC TACCCTGTTG CTCAGTGACA	1100
	AAGAATCACT CTGTGGCGGA GGCAGGCTTC AAGCTTGCAG CAATCCTCCT GCACCAGTTT	1160
55	CCTGAGTGCC AGACTTCCAG GTGTAAGCTA TGGCACTTAG CAGAACACTA GCTGAATCAA	1220
	TGAAGACACT GAGGTTCCAA GAGGGAACCT GAATTATGAA GGTGAGTCAG AATCCAGATT	1280
	TCCTGGCTCT ACCACTCTTA ACCTGTATCT GTTAGACCCC AAGCTCTGAG CTCATAGACA	1340
	AGCTAATTTA AAATGCTTTT TAATAAGCAG AAGGCTCAGT TAGTACGGGG TTCAGGATAC	1400
	TGCTTACTGG CAATATTTGA CTAGCCTCTA TTTTGTGTTGT TTTTAAAGG CCTACTGACT	1460

GTAGTGTAAT TTGTAGGAAA CATGTTGCTA TGTATACCCA TTTGAGGGTA ATAAAAATGT 1520
 TGGTAATTTT CAGCCAGCAC TTTCCAGGTA TTTCCCTTTT TATCCTTCAT 1570

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(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 214 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met	Ala	Cys	Asn	Cys	Gln	Leu	Met	Gln	Asp	Thr	Pro	Leu	Leu	Lys	Phe	1	5	10	15
Pro	Cys	Pro	Arg	Leu	Ile	Leu	Leu	Phe	Val	Leu	Leu	Ile	Arg	Leu	Ser	20	25	30	
Gln	Val	Ser	Ser	Ala	Asp	Phe	Ser	Thr	Pro	Asn	Ile	Thr	Glu	Ser	Gly	35	40	45	
Asn	Pro	Ser	Ala	Asp	Thr	Lys	Arg	Ile	Thr	Cys	Phe	Ala	Ser	Gly	Gly	50	55	60	
Phe	Pro	Lys	Pro	Arg	Phe	Ser	Trp	Leu	Glu	Asn	Gly	Arg	Glu	Leu	Pro	65	70	75	80
Gly	Ile	Asn	Thr	Thr	Ile	Ser	Gln	Asp	Pro	Glu	Ser	Glu	Leu	Tyr	Thr	85	90	95	
Ile	Ser	Ser	Gln	Leu	Asp	Phe	Asn	Thr	Thr	Arg	Asn	His	Thr	Ile	Lys	100	105	110	
Cys	Leu	Ile	Lys	Tyr	Gly	Asp	Ala	His	Val	Ser	Glu	Asp	Phe	Thr	Trp	115	120	125	
Glu	Lys	Pro	Pro	Glu	Asp	Pro	Pro	Asp	Ser	Lys	Asn	Thr	Leu	Val	Leu	130	135	140	
Phe	Gly	Ala	Gly	Phe	Gly	Ala	Val	Ile	Thr	Val	Val	Val	Ile	Val	Val	145	150	155	160
Ile	Ile	Lys	Cys	Phe	Cys	Lys	His	Gly	Leu	Ile	Tyr	His	Leu	Gln	Leu	165	170	175	
Thr	Ser	Ser	Ala	Lys	Asp	Phe	Arg	Asn	Leu	Ala	Leu	Pro	Trp	Leu	Cys	180	185	190	
Lys	His	Gly	Ser	Leu	Gly	Glu	Ala	Ser	Ala	Val	Ile	Cys	Arg	Ser	Thr	195	200	205	
Gln	Thr	Asn	Glu	Pro	Gln											210			

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1261 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 194..1135

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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AGNCCCNAGA TTATTTCTCC CTGTATAAGG GACGCCCAGG AGGCCTGGGG AGCGGACAAG      60
20  GCTCCTTTTA CTTTCTTCT TCTTCTATTT TTTTACCTT CTATTTT TTTT CTTCATGTTC      120
    CTGTGATCTT CGGGAATGCT GCTGTGCTTG TGTGTGTGGT CCCTGAGCGC CGAGGTGGAG      180
    AGGCACTGGT GAC ATG TAT GTC ATC AAG ACA TGT GCA ACC TGC ACC ATG      229
    Met Tyr Val Ile Lys Thr Cys Ala Thr Cys Thr Met
    1             5             10
    GGC TTG GCA ATC CTT ATC TTT GTG ACA GTC TTG CTG ATC TCA GAT GCT      277
    Gly Leu Ala Ile Leu Ile Phe Val Thr Val Leu Leu Ile Ser Asp Ala
    15             20             25
    GTT TCC GTG GAG ACG CAA GCT TAT TTC AAT GGG ACT GCA TAT CTG CCG      325
    Val Ser Val Glu Thr Gln Ala Tyr Phe Asn Gly Thr Ala Tyr Leu Pro
    30             35             40
35  TGC CCA TTT ACA AAG GCT CAA AAC ATA AGC CTG AGT GAG CTG GTA GTA      373
    Cys Pro Phe Thr Lys Ala Gln Asn Ile Ser Leu Ser Glu Leu Val Val
    45             50             55             60
    TTT TGG CAG GAC CAG CAA AAG TTG GTT CTG TAC GAG CAC TAT TTG GGC      421
    Phe Trp Gln Asp Gln Gln Lys Leu Val Leu Tyr Glu His Tyr Leu Gly
    65             70             75
    ACA GAG AAA CTT GAT AGT GTG AAT GCC AAG TAC CTG GGC CGC ACG AGC      469
    Thr Glu Lys Leu Asp Ser Val Asn Ala Lys Tyr Leu Gly Arg Thr Ser
    80             85             90
    TTT GAC AGG AAC AAC TGG ACT CTA CGA CTT CAC AAT GTT CAG ATC AAG      517
    Phe Asp Arg Asn Asn Trp Thr Leu Arg Leu His Asn Val Gln Ile Lys
    95             100             105
    GAC ATG GGC TCG TAT GAT TGT TTT ATA CAA AAA AAG CCA CCC ACA GGA      565
    Asp Met Gly Ser Tyr Asp Cys Phe Ile Gln Lys Lys Pro Pro Thr Gly
    110            115            120
55  TCA ATT ATC CTC CAA CAG ACA TTA ACA GAA CTG TCA GTG ATC GCC AAC      613
    Ser Ile Ile Leu Gln Gln Thr Leu Thr Glu Leu Ser Val Ile Ala Asn
    125            130            135            140

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	TTC AGT GAA CCT GAA ATA AAA CTG GCT CAG AAT GTA ACA GGA AAT TCT	661
	Phe Ser Glu Pro Glu Ile Lys Leu Ala Gln Asn Val Thr Gly Asn Ser	
	145 150 155	
5	GGC ATA AAT TTG ACC TGC ACG TCT AAG CAA GGT CAC CCG AAA CCT AAG	709
	Gly Ile Asn Leu Thr Cys Thr Ser Lys Gln Gly His Pro Lys Pro Lys	
	160 165 170	
10	AAG ATG TAT TTT CTG ATA ACT AAT TCA ACT AAT GAG TAT GGT GAT AAC	757
	Lys Met Tyr Phe Leu Ile Thr Asn Ser Thr Asn Glu Tyr Gly Asp Asn	
	175 180 185	
15	ATG CAG ATA TCA CAA GAT AAT GTC ACA GAA CTG TTC AGT ATC TCC AAC	805
	Met Gln Ile Ser Gln Asp Asn Val Thr Glu Leu Phe Ser Ile Ser Asn	
	190 195 200	
20	AGC CTC TCT CTT TCA TTC CCG GAT GGT GTG TGG CAT ATG ACC GTT GTG	853
	Ser Leu Ser Leu Ser Phe Pro Asp Gly Val Trp His Met Thr Val Val	
	205 210 215 220	
	TGT GTT CTG GAA ACG GAG TCA ATG AAG ATT TCC TCC AAA CCT CTC AAT	901
	Cys Val Leu Glu Thr Glu Ser Met Lys Ile Ser Ser Lys Pro Leu Asn	
	225 230 235	
25	TTC ACT CAA GAG TTT CCA TCT CCT CAA ACG TAT TGG AAG GAG ATT ACA	949
	Phe Thr Gln Glu Phe Pro Ser Pro Gln Thr Tyr Trp Lys Glu Ile Thr	
	240 245 250	
30	GCT TCA GTT ACT GTG GCC CTC CTC CTT GTG ATG CTG CTC ATC ATT GTA	997
	Ala Ser Val Thr Val Ala Leu Leu Leu Val Met Leu Leu Ile Ile Val	
	255 260 265	
35	TGT CAC AAG AAG CCG AAT CAG CCT AGC AGG CCC AGC AAC ACA GCC TCT	1045
	Cys His Lys Lys Pro Asn Gln Pro Ser Arg Pro Ser Asn Thr Ala Ser	
	270 275 280	
40	AAG TTA GAG CGG GAT AGT AAC GCT GAC AGA GAG ACT ATC AAC CTG AAG	1093
	Lys Leu Glu Arg Asp Ser Asn Ala Asp Arg Glu Thr Ile Asn Leu Lys	
	285 290 295 300	
	GAA CTT GAA CCC CAA ATT GCT TCA GCA AAA CCA AAT GCA GAG	1135
	Glu Leu Glu Pro Gln Ile Ala Ser Ala Lys Pro Asn Ala Glu	
	305 310	
45	TGAAGGCAGT GAGAGCCTGA GGAAAGAGTT AAAAATTGCT TTGCCTGAAA TAAGAAGTGC	1195
	AGAGTTTCTC AGAATTCAAA AATGTTCTCA GCTGATTGGA ATTCTACAGT TGAATAATTA	1255
50	AAGAAC	1261

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 314 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

5	Met	Tyr	Val	Ile	Lys	Thr	Cys	Ala	Thr	Cys	Thr	Met	Gly	Leu	Ala	Ile	1	5	10	15
	Leu	Ile	Phe	Val	Thr	Val	Leu	Leu	Ile	Ser	Asp	Ala	Val	Ser	Val	Glu	20	25	30	
10	Thr	Gln	Ala	Tyr	Phe	Asn	Gly	Thr	Ala	Tyr	Leu	Pro	Cys	Pro	Phe	Thr	35	40	45	
	Lys	Ala	Gln	Asn	Ile	Ser	Leu	Ser	Glu	Leu	Val	Val	Phe	Trp	Gln	Asp	50	55	60	
15	Gln	Gln	Lys	Leu	Val	Leu	Tyr	Glu	His	Tyr	Leu	Gly	Thr	Glu	Lys	Leu	65	70	75	80
	Asp	Ser	Val	Asn	Ala	Lys	Tyr	Leu	Gly	Arg	Thr	Ser	Phe	Asp	Arg	Asn	85	90	95	
20	Asn	Trp	Thr	Leu	Arg	Leu	His	Asn	Val	Gln	Ile	Lys	Asp	Met	Gly	Ser	100	105	110	
25	Tyr	Asp	Cys	Phe	Ile	Gln	Lys	Lys	Pro	Pro	Thr	Gly	Ser	Ile	Ile	Leu	115	120	125	
	Gln	Gln	Thr	Leu	Thr	Glu	Leu	Ser	Val	Ile	Ala	Asn	Phe	Ser	Glu	Pro	130	135	140	
30	Glu	Ile	Lys	Leu	Ala	Gln	Asn	Val	Thr	Gly	Asn	Ser	Gly	Ile	Asn	Leu	145	150	155	160
	Thr	Cys	Thr	Ser	Lys	Gln	Gly	His	Pro	Lys	Pro	Lys	Lys	Met	Tyr	Phe	165	170	175	
35	Leu	Ile	Thr	Asn	Ser	Thr	Asn	Glu	Tyr	Gly	Asp	Asn	Met	Gln	Ile	Ser	180	185	190	
40	Gln	Asp	Asn	Val	Thr	Glu	Leu	Phe	Ser	Ile	Ser	Asn	Ser	Leu	Ser	Leu	195	200	205	
	Ser	Phe	Pro	Asp	Gly	Val	Trp	His	Met	Thr	Val	Val	Cys	Val	Leu	Glu	210	215	220	
45	Thr	Glu	Ser	Met	Lys	Ile	Ser	Ser	Lys	Pro	Leu	Asn	Phe	Thr	Gln	Glu	225	230	235	240
	Phe	Pro	Ser	Pro	Gln	Thr	Tyr	Trp	Lys	Glu	Ile	Thr	Ala	Ser	Val	Thr	245	250	255	
50	Val	Ala	Leu	Leu	Leu	Val	Met	Leu	Leu	Ile	Ile	Val	Cys	His	Lys	Lys	260	265	270	
55	Pro	Asn	Gln	Pro	Ser	Arg	Pro	Ser	Asn	Thr	Ala	Ser	Lys	Leu	Glu	Arg	275	280	285	
	Asp	Ser	Asn	Ala	Asp	Arg	Glu	Thr	Ile	Asn	Leu	Lys	Glu	Leu	Glu	Pro	290	295	300	

Gln Ile Ala Ser Ala Lys Pro Asn Ala Glu
305 310

5 (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 223 base pairs
(B) TYPE: nucleic acid
10 (C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- 15 (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 194..223

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGNCCCNAGA TTATTTCTCC CTGTATAAGG GACGCCCAGG AGGCCTGGGG AGCGGACAAG 60
25 GCTCCTTTTA CTTTCTTCT TCTTCTATTT TTTTACCTT CTATTTTTTT CTTCATGTTT 120
CTGTGATCTT CGGGAATGCT GCTGTGCTTG TGTGTGTGGT CCCTGAGCGC CGAGGTGGAG 180
AGGCACTGGT GAC ATG TAT GTC ATC AAG ACA TGT GCA ACC TGC 223
30 Met Tyr Val Ile Lys Thr Cys Ala Thr Cys
1 5 10

35 (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

45 Met Tyr Val Ile Lys Thr Cys Ala Thr Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

- 50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1716 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 249..1166

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

	GAGTTTATA CCTCAATAGA CTCTTACTAG TTTCTCTTTT TCAGGTTGTG AAACCTCAACC	60
10	TTCAAAGACA CTCTGTTCCA TTTCTGTGGA CTAATAGGAT CATCTTTAGC ATCTGCCGGG	120
	TGGATGCCAT CCAGGCTTCT TTTTCTACAT CTCTGTTTCT CGATTTTGTG GAGCCTAGGA	180
	GGTGCCTAAG CTCCATTGGC TCTAGATTCC TGGCTTTCCC CATCATGTTC TCAAAGCAT	240
15	CTGAAGCT ATG GCT TGC AAT TGT CAG TTG ATG CAG GAT ACA CCA CTC CTC	290
	Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu	
	1 5 10	
20	AAG TTT CCA TGT CCA AGG CTC AAT CTT CTC TTT GTG CTG CTG ATT CGT	338
	Lys Phe Pro Cys Pro Arg Leu Asn Leu Leu Phe Val Leu Leu Ile Arg	
	15 20 25 30	
25	CTT TCA CAA GTG TCT TCA GAT GTT GAT GAA CAA CTG TCC AAG TCA GTG	386
	Leu Ser Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val	
	35 40 45	
30	AAA GAT AAG GTA TTG CTG CCT TGC CGT TAC AAC TCT CCT CAT GAA GAT	434
	Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp	
	50 55 60	
	GAG TCT GAA GAC CGA ATC TAC TGG CAA AAA CAT GAC AAA GTG GTG CTG	482
	Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu	
	65 70 75	
35	TCT GTC ATT GCT GGG AAA CTA AAA GTG TGG CCC GAG TAT AAG AAC CGG	530
	Ser Val Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg	
	80 85 90	
40	ACT TTA TAT GAC AAC ACT ACC TAC TCT CTT ATC ATC CTG GGC CTG GTC	578
	Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val	
	95 100 105 110	
45	CTT TCA GAC CGG GGC ACA TAC AGC TGT GTC GTT CAA AAG AAG GAA AGA	626
	Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg	
	115 120 125	
50	GGA ACG TAT GAA GTT AAA CAC TTG GCT TTA GTA AAG TTG TCC ATC AAA	674
	Gly Thr Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys	
	130 135 140	
	GCT GAC TTC TCT ACC CCC AAC ATA ACT GAG TCT GGA AAC CCA TCT GCA	722
	Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala	
	145 150 155	
55	GAC ACT AAA AGG ATT ACC TGC TTT GCT TCC GGG GGT TTC CCA AAG CCT	770
	Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser Gly Phe Pro Lys Pro	
	160 165 170	

	CGC TTC TCT TGG TTG GAA AAT GGA AGA GAA TTA CCT GGC ATC AAT ACG	818
	Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr	
	175 180 185 190	
5	ACA ATT TCC CAG GAT CCT GAA TCT GAA TTG TAC ACC ATT AGT AGC CAA	866
	Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln	
	195 200 205	
10	CTA GAT TTC AAT ACG ACT CGC AAC CAC ACC ATT AAG TGT CTC ATT AAA	914
	Leu Asp Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys	
	210 215 220	
15	TAT GGA GAT GCT CAC GTG TCA GAG GAC TTC ACC TGG GAA AAA CCC CCA	962
	Tyr Gly Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro	
	225 230 235	
20	GAA GAC CCT CCT GAT AGC AAG AAC ACA CTT GTG CTC TTT GGG GCA GGA	1010
	Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly	
	240 245 250	
	TTC GGC GCA GTA ATA ACA GTC GTC GTC ATC GTT GTC ATC ATC AAA TGC	1058
	Phe Gly Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys	
	255 260 265 270	
25	TTC TGT AAG CAC AGA AGC TGT TTC AGA AGA AAT GAG GCA AGC AGA GAA	1106
	Phe Cys Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu	
	275 280 285	
30	ACA AAC AAC AGC CTT ACC TTC GGG CCT GAA GAA GCA TTA GCT GAA CAG	1154
	Thr Asn Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln	
	290 295 300	
35	ACC GTC TTC CTT TAGTTCTTCT CTGTCCATGT GGGATACATG GTATTATGTG	1206
	Thr Val Phe Leu	
	305	
	GCTCATGAGG TACAATCTTT CTTTCAGCAC CGTGCTAGCT GATCTTTCGG ACAACTTGAC	1266
40	ACAAGATAGA GTTAACTGGG AAGAGAAAGC CTTGAATGAG GATTTCTTTC CATCAGGAAG	1326
	CTACGGGCAA GTTTGCTGGG CCTTTGATTG CTTGATGACT GAAGTGGAAA GGCTGAGCCC	1386
	ACTGTGGGTG GTGCTAGCCC TGGGCAGGGG CAGGTGACCC TGGGTGGTAT AAGAAAAAGA	1446
45	GCTGTCACTA AAAGGAGAGG TGCCTAGTCT TACTGCAACT TGATATGTCA TGTTTGTTG	1506
	GTGTCTGTGG GAGGCCTGCC CTTTCTGAA GAGAAGTGGT GGGAGAGTGG ATGGGGTGGG	1566
50	GGCAGAGGAA AAGTGGGGGA GAGGCCTGG GAGGAGAGGA GGGAGGGGGA CGGGGTGGGG	1626
	GTGGGGAAAA CTATGGTTGG GATGTAAAAA CGGATAATAA TATAAATATT AAATAAAAAG	1686
	AGAGTATTGA GCAAAAAAAA AAAAAAAAAA	1716

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(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 306 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met	Ala	Cys	Asn	Cys	Gln	Leu	Met	Gln	Asp	Thr	Pro	Leu	Leu	Lys	Phe	
1				5					10					15		
Pro	Cys	Pro	Arg	Leu	Ile	Leu	Leu	Phe	Val	Leu	Leu	Ile	Arg	Leu	Ser	
			20					25					30			
Gln	Val	Ser	Ser	Asp	Val	Asp	Glu	Gln	Leu	Ser	Lys	Ser	Val	Lys	Asp	
		35					40					45				
Lys	Val	Leu	Leu	Pro	Cys	Arg	Tyr	Asn	Ser	Pro	His	Glu	Asp	Glu	Ser	
	50					55					60					
Glu	Asp	Arg	Ile	Tyr	Trp	Gln	Lys	His	Asp	Lys	Val	Val	Leu	Ser	Val	
65					70					75					80	
Ile	Ala	Gly	Lys	Leu	Lys	Val	Trp	Pro	Glu	Tyr	Lys	Asn	Arg	Thr	Leu	
				85					90					95		
Tyr	Asp	Asn	Thr	Thr	Tyr	Ser	Leu	Ile	Ile	Leu	Gly	Leu	Val	Leu	Ser	
			100					105					110			
Asp	Arg	Gly	Thr	Tyr	Ser	Cys	Val	Val	Gln	Lys	Lys	Glu	Arg	Gly	Thr	
		115					120					125				
Tyr	Glu	Val	Lys	His	Leu	Ala	Leu	Val	Lys	Leu	Ser	Ile	Lys	Ala	Asp	
	130					135					140					
Phe	Ser	Thr	Pro	Asn	Ile	Thr	Glu	Ser	Gly	Asn	Pro	Ser	Ala	Asp	Thr	
145					150					155				160		
Lys	Arg	Ile	Thr	Cys	Phe	Ala	Ser	Gly	Gly	Phe	Pro	Lys	Pro	Arg	Phe	
				165					170					175		
Ser	Trp	Leu	Glu	Asn	Gly	Arg	Glu	Leu	Pro	Gly	Ile	Asn	Thr	Thr	Ile	
		180						185					190			
Ser	Gln	Asp	Pro	Glu	Ser	Glu	Leu	Tyr	Thr	Ile	Ser	Ser	Gln	Leu	Asp	
		195					200					205				
Phe	Asn	Thr	Thr	Arg	Asn	His	Thr	Ile	Lys	Cys	Leu	Ile	Lys	Tyr	Gly	
	210					215					220					
Asp	Ala	His	Val	Ser	Glu	Asp	Phe	Thr	Trp	Glu	Lys	Pro	Pro	Glu	Asp	
225					230					235				240		
Pro	Pro	Asp	Ser	Lys	Asn	Thr	Leu	Val	Leu	Phe	Gly	Ala	Gly	Phe	Gly	
				245					250					255		
Ala	Val	Ile	Thr	Val	Val	Val	Ile	Val	Val	Ile	Ile	Lys	Cys	Phe	Cys	
			260					265					270			

55

Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn
 275 280 285

5 Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln Thr Val
 290 295 300

Phe Leu
 305

10 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1491 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 318..1181

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCAAAGAAAA AGTGATTTGT CATTGCTTTA TAGACTGTAA GAAGAGAACA TCTCAGAAGT 60
 30 GGAGTCTTAC CCTGAAATCA AAGGATTTAA AGAAAAAGTG GAATTTTTCT TCAGCAAGCT 120
 GTGAAACTAA ATCCACAACC TTTGGAGACC CAGGAACACC CTCCAATCTC TGTGTGTTTT 180
 35 GTAAACATCA CTGGAGGGTC TTCTACGTGA GCAATTGGAT TGTCATCAGC CCTGCCTGTT 240
 TTGCACCTGG GAAGTGCCCT GGTCTTACTT GGGTCCAAAT TGTTGGCTTT CACTTTTGAC 300
 CCTAAGCATC TGAAGCC ATG GGC CAC ACA CGG AGG CAG GGA ACA TCA CCA 350
 40 Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro
 1 5 10
 TCC AAG TGT CCA TAC CTG AAT TTC TTT CAG CTC TTG GTG CTG GCT GGT 398
 Ser Lys Cys Pro Tyr Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly
 15 20 25
 45 CTT TCT CAC TTC TGT TCA GGT GTT ATC CAC GTG ACC AAG GAA GTG AAA 446
 Leu Ser His Phe Cys Ser Gly Val Ile His Val Thr Lys Glu Val Lys
 30 35 40
 50 GAA GTG GCA ACG CTG TCC TGT GGT CAC AAT GTT TCT GTT GAA GAG CTG 494
 Glu Val Ala Thr Leu Ser Cys Gly His Asn Val Ser Val Glu Glu Leu
 45 50 55
 55 GCA CAA ACT CGC ATC TAC TGG CAA AAG GAG AAG AAA ATG GTG CTG ACT 542
 Ala Gln Thr Arg Ile Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr
 60 65 70 75

AAGCTGAACA GTTACAAGAT GGCTGGCATC CCTCTCCTTT CTCCCATAT GCAATTTGCT 1401
 5 TAATGTAACC TCTTCTTTTG CCATGTTTCC ATTCTGCCAT CTTGAATTGT CTTGTCAGCC 1461
 AATTCATTAT CTATTAAACA CTAATTTGAG 1491

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 288 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

20 Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr
 1 5 10 15
 Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys
 20 25 30
 25 Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu
 35 40 45
 30 Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile
 50 55 60
 Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp
 65 70 75 80
 35 Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr
 85 90 95
 Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly
 100 105 110
 40 Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg
 115 120 125
 45 Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr
 130 135 140
 Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile
 145 150 155 160
 50 Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu
 165 170 175
 Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp
 180 185 190
 55 Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met
 195 200 205

Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg
 210 215 220
 Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro
 5 225 230 235 240
 Asp Asn Leu Leu Pro Ser Trp Ala Ile Thr Leu Ile Ser Val Asn Gly
 245 250 255
 10 Ile Phe Val Ile Cys Cys Leu Thr Tyr Cys Phe Ala Pro Arg Cys Arg
 260 265 270
 Glu Arg Arg Arg Asn Glu Arg Leu Arg Arg Glu Ser Val Arg Pro Val
 15 275 280 285

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 1151 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- 30 (A) NAME/KEY: CDS
 (B) LOCATION: 99..1025

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

35 GGAGCAAGCA GACGCGTAAG AGTGGCTCCT GTAGGCAGCA CGGACTTGAA CAACCAGACT 60
 CCTGTAGACG TGTTCAGAA CTTACGGAAG CACCCACG ATG GAC CCC AGA TGC 113
 Met Asp Pro Arg Cys
 1 5
 40 ACC ATG GGC TTG GCA ATC CTT ATC TTT GTG ACA GTC TTG CTG ATC TCA 161
 Thr Met Gly Leu Ala Ile Leu Ile Phe Val Thr Val Leu Leu Ile Ser
 10 15 20
 45 GAT GCT GTT TCC GTG GAG ACG CAA GCT TAT TTC AAT GGG ACT GCA TAT 209
 Asp Ala Val Ser Val Glu Thr Gln Ala Tyr Phe Asn Gly Thr Ala Tyr
 25 30 35
 50 CTG CCG TGC CCA TTT ACA AAG GCT CAA AAC ATA AGC CTG AGT GAG CTG 257
 Leu Pro Cys Pro Phe Thr Lys Ala Gln Asn Ile Ser Leu Ser Glu Leu
 40 45 50
 55 GTA GTA TTT TGG CAG GAC CAG CAA AAG TTG GTT CTG TAC GAG CAC TAT 305
 Val Val Phe Trp Gln Asp Gln Gln Lys Leu Val Leu Tyr Glu His Tyr
 55 60 65
 TTG GGC ACA GAG AAA CTT GAT AGT GTG AAT GCC AAG TAC CTG GGC CGC 353
 Leu Gly Thr Glu Lys Leu Asp Ser Val Asn Ala Lys Tyr Leu Gly Arg
 70 75 80 85

	ACG AGC TTT GAC AGG AAC AAC TGG ACT CTA CGA CTT CAC AAT GTT CAG	401
	Thr Ser Phe Asp Arg Asn Asn Trp Thr Leu Arg Leu His Asn Val Gln	
	90 95 100	
5	ATC AAG GAC ATG GGC TCG TAT GAT TGT TTT ATA CAA AAA AAG CCA CCC	449
	Ile Lys Asp Met Gly Ser Tyr Asp Cys Phe Ile Gln Lys Lys Pro Pro	
	105 110 115	
10	ACA GGA TCA ATT ATC CTC CAA CAG ACA TTA ACA GAA CTG TCA GTG ATC	497
	Thr Gly Ser Ile Ile Leu Gln Gln Thr Leu Thr Glu Leu Ser Val Ile	
	120 125 130	
15	GCC AAC TTC AGT GAA CCT GAA ATA AAA CTG GCT CAG AAT GTA ACA GGA	545
	Ala Asn Phe Ser Glu Pro Glu Ile Lys Leu Ala Gln Asn Val Thr Gly	
	135 140 145	
20	AAT TCT GGC ATA AAT TTG ACC TGC ACG TCT AAG CAA GGT CAC CCG AAA	593
	Asn Ser Gly Ile Asn Leu Thr Cys Thr Ser Lys Gln Gly His Pro Lys	
	150 155 160 165	
25	CCT AAG AAG ATG TAT TTT CTG ATA ACT AAT TCA ACT AAT GAG TAT GGT	641
	Pro Lys Lys Met Tyr Phe Leu Ile Thr Asn Ser Thr Asn Glu Tyr Gly	
	170 175 180	
30	GAT AAC ATG CAG ATA TCA CAA GAT AAT GTC ACA GAA CTG TTC AGT ATC	689
	Asp Asn Met Gln Ile Ser Gln Asp Asn Val Thr Glu Leu Phe Ser Ile	
	185 190 195	
35	TCC AAC AGC CTC TCT CTT TCA TTC CCG GAT GGT GTG TGG CAT ATG ACC	737
	Ser Asn Ser Leu Ser Leu Ser Phe Pro Asp Gly Val Trp His Met Thr	
	200 205 210	
40	GTT GTG TGT GTT CTG GAA ACG GAG TCA ATG AAG ATT TCC TCC AAA CCT	785
	Val Val Cys Val Leu Glu Thr Glu Ser Met Lys Ile Ser Ser Lys Pro	
	215 220 225	
45	CTC AAT TTC ACT CAA GAG TTT CCA TCT CCT CAA ACG TAT TGG AAG GAG	833
	Leu Asn Phe Thr Gln Glu Phe Pro Ser Pro Gln Thr Tyr Trp Lys Glu	
	230 235 240 245	
50	ATT ACA GCT TCA GTT ACT GTG GCC CTC CTC CTT GTG ATG CTG CTC ATC	881
	Ile Thr Ala Ser Val Thr Val Ala Leu Leu Leu Val Met Leu Leu Ile	
	250 255 260	
55	ATT GTA TGT CAC AAG AAG CCG AAT CAG CCT AGC AGG CCC AGC AAC ACA	929
	Ile Val Cys His Lys Lys Pro Asn Gln Pro Ser Arg Pro Ser Asn Thr	
	265 270 275	
60	GCC TCT AAG TTA GAG CGG GAT AGT AAC GCT GAC AGA GAG ACT ATC AAC	977
	Ala Ser Lys Leu Glu Arg Asp Ser Asn Ala Asp Arg Glu Thr Ile Asn	
	280 285 290	
65	CTG AAG GAA CTT GAA CCC CAA ATT GCT TCA GCA AAA CCA AAT GCA GAG	1025
	Leu Lys Glu Leu Glu Pro Gln Ile Ala Ser Ala Lys Pro Asn Ala Glu	
	295 300 305	
	TGAAGGCAGT GAGAGCCTGA GGAAAGAGTT AAAAATTGCT TTGCCTGAAA TAAGAAGTGC	1085

AGAGTTTCTC AGAATTCAAA AATGTTCTCA GCTGATTGGA ATTCTACAGT TGAATAATTA 1145
AAGAAC 1151

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(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH: 309 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

	Met	Asp	Pro	Arg	Cys	Thr	Met	Gly	Leu	Ala	Ile	Leu	Ile	Phe	Val	Thr	
	1				5					10					15		
20	Val	Leu	Leu	Ile	Ser	Asp	Ala	Val	Ser	Val	Glu	Thr	Gln	Ala	Tyr	Phe	
				20					25					30			
	Asn	Gly	Thr	Ala	Tyr	Leu	Pro	Cys	Pro	Phe	Thr	Lys	Ala	Gln	Asn	Ile	
			35					40					45				
25	Ser	Leu	Ser	Glu	Leu	Val	Val	Phe	Trp	Gln	Asp	Gln	Gln	Lys	Leu	Val	
		50					55					60					
	Leu	Tyr	Glu	His	Tyr	Leu	Gly	Thr	Glu	Lys	Leu	Asp	Ser	Val	Asn	Ala	
30		65				70				75						80	
	Lys	Tyr	Leu	Gly	Arg	Thr	Ser	Phe	Asp	Arg	Asn	Asn	Trp	Thr	Leu	Arg	
					85					90					95		
35	Leu	His	Asn	Val	Gln	Ile	Lys	Asp	Met	Gly	Ser	Tyr	Asp	Cys	Phe	Ile	
				100					105					110			
	Gln	Lys	Lys	Pro	Pro	Thr	Gly	Ser	Ile	Ile	Leu	Gln	Gln	Thr	Leu	Thr	
			115					120					125				
40	Glu	Leu	Ser	Val	Ile	Ala	Asn	Phe	Ser	Glu	Pro	Glu	Ile	Lys	Leu	Ala	
		130					135					140					
	Gln	Asn	Val	Thr	Gly	Asn	Ser	Gly	Ile	Asn	Leu	Thr	Cys	Thr	Ser	Lys	
45		145				150				155						160	
	Gln	Gly	His	Pro	Lys	Pro	Lys	Lys	Met	Tyr	Phe	Leu	Ile	Thr	Asn	Ser	
					165				170						175		
50	Thr	Asn	Glu	Tyr	Gly	Asp	Asn	Met	Gln	Ile	Ser	Gln	Asp	Asn	Val	Thr	
				180				185						190			
	Glu	Leu	Phe	Ser	Ile	Ser	Asn	Ser	Leu	Ser	Leu	Ser	Phe	Pro	Asp	Gly	
			195				200						205				
55	Val	Trp	His	Met	Thr	Val	Val	Cys	Val	Leu	Glu	Thr	Glu	Ser	Met	Lys	
		210				215						220					

Ile Ser Ser Lys Pro Leu Asn Phe Thr Gln Glu Phe Pro Ser Pro Gln
 225 230 235 240

5 Thr Tyr Trp Lys Glu Ile Thr Ala Ser Val Thr Val Ala Leu Leu Leu
 245 250 255

Val Met Leu Leu Ile Ile Val Cys His Lys Lys Pro Asn Gln Pro Ser
 260 265 270

10 Arg Pro Ser Asn Thr Ala Ser Lys Leu Glu Arg Asp Ser Asn Ala Asp
 275 280 285

Arg Glu Thr Ile Asn Leu Lys Glu Leu Glu Pro Gln Ile Ala Ser Ala
 290 295 300

15 Lys Pro Asn Ala Glu
 305

(2) INFORMATION FOR SEQ ID NO:22:

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1120 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 107..1093

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CACAGGGTGA AAGCTTTGCT TCTCTGCTGC TGTAACAGGG ACTAGCACAG ACACACGGAT 60

40 GAGTGGGGTTC ATTTCCAGAT ATTAGGTCAC AGCAGAAGCA GCCAAA ATG GAT CCC 115
 Met Asp Pro
 1

45 CAG TGC ACT ATG GGA CTG AGT AAC ATT CTC TTT GTG ATG GCC TTC CTG 163
 Gln Cys Thr Met Gly Leu Ser Asn Ile Leu Phe Val Met Ala Phe Leu
 5 10 15

CTC TCT GGT GCT GCT CCT CTG AAG ATT CAA GCT TAT TTC AAT GAG ACT 211
 Leu Ser Gly Ala Ala Pro Leu Lys Ile Gln Ala Tyr Phe Asn Glu Thr
 20 25 30 35

50 GCA GAC CTG CCA TGC CAA TTT GCA AAC TCT CAA AAC CAA AGC CTG AGT 259
 Ala Asp Leu Pro Cys Gln Phe Ala Asn Ser Gln Asn Gln Ser Leu Ser
 40 45 50

55 GAG CTA GTA GTA TTT TGG CAG GAC CAG GAA AAC TTG GTT CTG AAT GAG 307
 Glu Leu Val Val Phe Trp Gln Asp Gln Glu Asn Leu Val Leu Asn Glu
 55 60 65

	GTA	TAC	TTA	GGC	AAA	GAG	AAA	TTT	GAC	AGT	GTT	CAT	TCC	AAG	TAT	ATG	355
	Val	Tyr	Leu	Gly	Lys	Glu	Lys	Phe	Asp	Ser	Val	His	Ser	Lys	Tyr	Met	
			70					75					80				
5	GGC	CGC	ACA	AGT	TTT	GAT	TCG	GAC	AGT	TGG	ACC	CTG	AGA	CTT	CAC	AAT	403
	Gly	Arg	Thr	Ser	Phe	Asp	Ser	Asp	Ser	Trp	Thr	Leu	Arg	Leu	His	Asn	
		85					90					95					
10	CTT	CAG	ATC	AAG	GAC	AAG	GGC	TTG	TAT	CAA	TGT	ATC	ATC	CAT	CAC	AAA	451
	Leu	Gln	Ile	Lys	Asp	Lys	Gly	Leu	Tyr	Gln	Cys	Ile	Ile	His	His	Lys	
	100					105					110					115	
15	AAG	CCC	ACA	GGA	ATG	ATT	CGC	ATC	CAC	CAG	ATG	AAT	TCT	GAA	CTG	TCA	499
	Lys	Pro	Thr	Gly	Met	Ile	Arg	Ile	His	Gln	Met	Asn	Ser	Glu	Leu	Ser	
					120					125					130		
20	GTG	CTT	GCT	AAC	TTC	AGT	CAA	CCT	GAA	ATA	GTA	CCA	ATT	TCT	AAT	ATA	547
	Val	Leu	Ala	Asn	Phe	Ser	Gln	Pro	Glu	Ile	Val	Pro	Ile	Ser	Asn	Ile	
				135					140					145			
	ACA	GAA	AAT	GTG	TAC	ATA	AAT	TTG	ACC	TGC	TCA	TCT	ATA	CAC	GGT	TAC	595
	Thr	Glu	Asn	Val	Tyr	Ile	Asn	Leu	Thr	Cys	Ser	Ser	Ile	His	Gly	Tyr	
			150					155					160				
25	CCA	GAA	CCT	AAG	AAG	ATG	AGT	GTT	TTG	CTA	AGA	ACC	AAG	AAT	TCA	ACT	643
	Pro	Glu	Pro	Lys	Lys	Met	Ser	Val	Leu	Leu	Arg	Thr	Lys	Asn	Ser	Thr	
		165					170					175					
30	ATC	GAG	TAT	GAT	GGT	ATT	ATG	CAG	AAA	TCT	CAA	GAT	AAT	GTC	ACA	GAA	691
	Ile	Glu	Tyr	Asp	Gly	Ile	Met	Gln	Lys	Ser	Gln	Asp	Asn	Val	Thr	Glu	
	180					185					190					195	
35	CTG	TAC	GAC	GTT	TCC	ATC	AGC	TTG	TCT	GTT	TCA	TTC	CCT	GAT	GTT	ACG	739
	Leu	Tyr	Asp	Val	Ser	Ile	Ser	Leu	Ser	Val	Ser	Phe	Pro	Asp	Val	Thr	
					200					205					210		
40	AGC	AAT	ATG	ACC	ATC	TTC	TGT	ATT	CTG	GAA	ACT	GAC	AAG	ACG	CGG	CTT	787
	Ser	Asn	Met	Thr	Ile	Phe	Cys	Ile	Leu	Glu	Thr	Asp	Lys	Thr	Arg	Leu	
				215					220					225			
	TTA	TCT	TCA	CCT	TTC	TCT	ATA	GAG	CTT	GAG	GAC	CCT	CAG	CCT	CCC	CCA	835
	Leu	Ser	Ser	Pro	Phe	Ser	Ile	Glu	Leu	Glu	Asp	Pro	Gln	Pro	Pro	Pro	
			230					235					240				
45	GAC	CAC	ATT	CCT	TGG	ATT	ACA	GCT	GTA	CTT	CCA	ACA	GTT	ATT	ATA	TGT	883
	Asp	His	Ile	Pro	Trp	Ile	Thr	Ala	Val	Leu	Pro	Thr	Val	Ile	Ile	Cys	
		245					250					255					
50	GTG	ATG	GTT	TTC	TGT	CTA	ATT	CTA	TGG	AAA	TGG	AAG	AAG	AAG	AAG	CGG	931
	Val	Met	Val	Phe	Cys	Leu	Ile	Leu	Trp	Lys	Trp	Lys	Lys	Lys	Lys	Arg	
	260					265				270						275	
55	CCT	CGC	AAC	TCT	TAT	AAA	TGT	GGA	ACC	AAC	ACA	ATG	GAG	AGG	GAA	GAG	979
	Pro	Arg	Asn	Ser	Tyr	Lys	Cys	Gly	Thr	Asn	Thr	Met	Glu	Arg	Glu	Glu	
					280					285					290		
	AGT	GAA	CAG	ACC	AAG	AAA	AGA	GAA	AAA	ATC	CAT	ATA	CCT	GAA	AGA	TCT	1027
	Ser	Glu	Gln	Thr	Lys	Lys	Arg	Glu	Lys	Ile	His	Ile	Pro	Glu	Arg	Ser	
				295					300					305			

GAT GAA GCC CAG CGT GTT TTT AAA AGT TCG AAG ACA TCT TCA TGC GAC 1075
Asp Glu Ala Gln Arg Val Phe Lys Ser Ser Lys Thr Ser Ser Cys Asp
310 315 320

5

AAA AGT GAT ACA TGT TTT TAATTAAAGA GTAAAGCCCA AAAAAAA 1120
Lys Ser Asp Thr Cys Phe
325

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(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 329 amino acids

15

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Asp Pro Gln Cys Thr Met Gly Leu Ser Asn Ile Leu Phe Val Met
1 5 10 15

25 Ala Phe Leu Leu Ser Gly Ala Ala Pro Leu Lys Ile Gln Ala Tyr Phe
20 25 30

Asn Glu Thr Ala Asp Leu Pro Cys Gln Phe Ala Asn Ser Gln Asn Gln
35 40 45

30

Ser Leu Ser Glu Leu Val Val Phe Trp Gln Asp Gln Glu Asn Leu Val
50 55 60

35 Leu Asn Glu Val Tyr Leu Gly Lys Glu Lys Phe Asp Ser Val His Ser
 65 70 75 80

Lys Tyr Met Gly Arg Thr Ser Phe Asp Ser Asp Ser Trp Thr Leu Arg
85 90 95

40 Leu His Asn Leu Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys Ile Ile
 100 105 110

His His Lys Lys Pro Thr Gly Met Ile Arg Ile His Gln Met Asn Ser
115 120 125

45

Glu Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Val Pro Ile
130 135 140

50 Ser Asn Ile Thr Glu Asn Val Tyr Ile Asn Leu Thr Cys Ser Ser Ile
145 150 155 160

His Gly Tyr Pro Glu Pro Lys Lys Met Ser Val Leu Leu Arg Thr Lys
165 170 175

55 Asn Ser Thr Ile Glu Tyr Asp Gly Ile Met Gln Lys Ser Gln Asp Asn
 180 185 190

Val Thr Glu Leu Tyr Asp Val Ser Ile Ser Leu Ser Val Ser Phe Pro
195 200 205

Asp Val Thr Ser Asn Met Thr Ile Phe Cys Ile Leu Glu Thr Asp Lys
 210 215 220
 5 Thr Arg Leu Leu Ser Ser Pro Phe Ser Ile Glu Leu Glu Asp Pro Gln
 225 230 235 240
 Pro Pro Pro Asp His Ile Pro Trp Ile Thr Ala Val Leu Pro Thr Val
 245 250 255
 10 Ile Ile Cys Val Met Val Phe Cys Leu Ile Leu Trp Lys Trp Lys Lys
 260 265 270
 Lys Lys Arg Pro Arg Asn Ser Tyr Lys Cys Gly Thr Asn Thr Met Glu
 15 275 280 285
 Arg Glu Glu Ser Glu Gln Thr Lys Lys Arg Glu Lys Ile His Ile Pro
 290 295 300
 20 Glu Arg Ser Asp Glu Ala Gln Arg Val Phe Lys Ser Ser Lys Thr Ser
 305 310 315 320
 Ser Cys Asp Lys Ser Asp Thr Cys Phe
 325

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1161 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 148..1134

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

45 AGGAGCCTTA GGAGGTACGG GGAGCTCGCA AATACTCCTT TTGGTTTATT CTTACCACCT 60
 TGCTTCTGTG TTCCTTGGGA ATGCTGCTGT GCTTATGCAT CTGGTCTCTT TTTGGAGCTA 120
 CAGTGGACAG GCATTTGTGA CAGCACT ATG GAT CCC CAG TGC ACT ATG GGA 171
 50 Met Asp Pro Gln Cys Thr Met Gly
 1 5
 CTG AGT AAC ATT CTC TTT GTG ATG GCC TTC CTG CTC TCT GGT GCT GCT 219
 Leu Ser Asn Ile Leu Phe Val Met Ala Phe Leu Leu Ser Gly Ala Ala
 10 15 20
 55 CCT CTG AAG ATT CAA GCT TAT TTC AAT GAG ACT GCA GAC CTG CCA TGC 267
 Pro Leu Lys Ile Gln Ala Tyr Phe Asn Glu Thr Ala Asp Leu Pro Cys
 25 30 35 40

	CAA TTT GCA AAC TCT CAA AAC CAA AGC CTG AGT GAG CTA GTA GTA TTT	315
	Gln Phe Ala Asn Ser Gln Asn Gln Ser Leu Ser Glu Leu Val Val Phe	
	45 50 55	
5	TGG CAG GAC CAG GAA AAC TTG GTT CTG AAT GAG GTA TAC TTA GGC AAA	363
	Trp Gln Asp Gln Glu Asn Leu Val Leu Asn Glu Val Tyr Leu Gly Lys	
	60 65 70	
10	GAG AAA TTT GAC AGT GTT CAT TCC AAG TAT ATG GGC CGC ACA AGT TTT	411
	Glu Lys Phe Asp Ser Val His Ser Lys Tyr Met Gly Arg Thr Ser Phe	
	75 80 85	
15	GAT TCG GAC AGT TGG ACC CTG AGA CTT CAC AAT CTT CAG ATC AAG GAC	459
	Asp Ser Asp Ser Trp Thr Leu Arg Leu His Asn Leu Gln Ile Lys Asp	
	90 95 100	
20	AAG GGC TTG TAT CAA TGT ATC ATC CAT CAC AAA AAG CCC ACA GGA ATG	507
	Lys Gly Leu Tyr Gln Cys Ile Ile His His Lys Lys Pro Thr Gly Met	
	105 110 115 120	
	ATT CGC ATC CAC CAG ATG AAT TCT GAA CTG TCA GTG CTT GCT AAC TTC	555
	Ile Arg Ile His Gln Met Asn Ser Glu Leu Ser Val Leu Ala Asn Phe	
	125 130 135	
25	AGT CAA CCT GAA ATA GTA CCA ATT TCT AAT ATA ACA GAA AAT GTG TAC	603
	Ser Gln Pro Glu Ile Val Pro Ile Ser Asn Ile Thr Glu Asn Val Tyr	
	140 145 150	
30	ATA AAT TTG ACC TGC TCA TCT ATA CAC GGT TAC CCA GAA CCT AAG AAG	651
	Ile Asn Leu Thr Cys Ser Ser Ile His Gly Tyr Pro Glu Pro Lys Lys	
	155 160 165	
35	ATG AGT GTT TTG CTA AGA ACC AAG AAT TCA ACT ATC GAG TAT GAT GGT	699
	Met Ser Val Leu Leu Arg Thr Lys Asn Ser Thr Ile Glu Tyr Asp Gly	
	170 175 180	
40	ATT ATG CAG AAA TCT CAA GAT AAT GTC ACA GAA CTG TAC GAC GTT TCC	747
	Ile Met Gln Lys Ser Gln Asp Asn Val Thr Glu Leu Tyr Asp Val Ser	
	185 190 195 200	
	ATC AGC TTG TCT GTT TCA TTC CCT GAT GTT ACG AGC AAT ATG ACC ATC	795
	Ile Ser Leu Ser Val Ser Phe Pro Asp Val Thr Ser Asn Met Thr Ile	
	205 210 215	
45	TTC TGT ATT CTG GAA ACT GAC AAG ACG CGG CTT TTA TCT TCA CCT TTC	843
	Phe Cys Ile Leu Glu Thr Asp Lys Thr Arg Leu Leu Ser Ser Pro Phe	
	220 225 230	
50	TCT ATA GAG CTT GAG GAC CCT CAG CCT CCC CCA GAC CAC ATT CCT TGG	891
	Ser Ile Glu Leu Glu Asp Pro Gln Pro Pro Pro Asp His Ile Pro Trp	
	235 240 245	
55	ATT ACA GCT GTA CTT CCA ACA GTT ATT ATA TGT GTG ATG GTT TTC TGT	939
	Ile Thr Ala Val Leu Pro Thr Val Ile Ile Cys Val Met Val Phe Cys	
	250 255 260	
	CTA ATT CTA TGG AAA TGG AAG AAG AAG AAG CGG CCT CGC AAC TCT TAT	987
	Leu Ile Leu Trp Lys Trp Lys Lys Lys Lys Arg Pro Arg Asn Ser Tyr	
	265 270 275 280	

	AAA TGT GGA ACC AAC ACA ATG GAG AGG GAA GAG AGT GAA CAG ACC AAG	1035
	Lys Cys Gly Thr Asn Thr Met Glu Arg Glu Glu Ser Glu Gln Thr Lys	
	285 290 295	
5	AAA AGA GAA AAA ATC CAT ATA CCT GAA AGA TCT GAT GAA GCC CAG CGT	1083
	Lys Arg Glu Lys Ile His Ile Pro Glu Arg Ser Asp Glu Ala Gln Arg	
	300 305 310	
10	GTT TTT AAA AGT TCG AAG ACA TCT TCA TGC GAC AAA AGT GAT ACA TGT	1131
	Val Phe Lys Ser Ser Lys Thr Ser Ser Cys Asp Lys Ser Asp Thr Cys	
	315 320 325	
15	TTT TAATTAAAGA GTAAAGCCCA AAAAAAA	1161
	Phe	
20	(2) INFORMATION FOR SEQ ID NO:25:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 629 base pairs	
	(B) TYPE: nucleic acid	
25	(C) STRANDEDNESS: double	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
30	(ix) FEATURE:	
	(A) NAME/KEY: CDS	
	(B) LOCATION: 1..96	
35	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:	
	AGA AGC TGT TTC AGA AGA AAT GAG GCA AGC AGA GAA ACA AAC AAC AGC	48
	Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn Asn Ser	
	1 5 10 15	
40	CTT ACC TTC GGG CCT GAA GAA GCA TTA GCT GAA CAG ACC GTC TTC CTT	96
	Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln Thr Val Phe Leu	
	20 25 30	
45	TAGTTCTTCT CTGTCCATGT GGGATACATG GTATTATGTG GCTCATGAGG TACAATCTTT	156
	CTTTCAGCAC CGTGCTAGCT GATCTTTCGG ACAACTTGAC ACAAGATAGA GTTAAGTGGG	216
	AAGAGAAAGC CTTGAATGAG GATTTCTTTC CATCAGGAAG CTACGGGCAA GTTTGCTGGG	276
50	CCTTTGATTG CTTGATGACT GAAGTGGAAG GGCTGAGCCC ACTGTGGGTG GTGCTAGAAA	336
	TGGGCAGGGG CAGGTGACCC TGGGTGGTAT AAGAAAAAGA GCTGTCACTA AAAGGAGAGG	396
55	TGCCTAGTCT TACTGCAACT TGATATGTCA TGTTTGTTG GTGTCTGTGG GAGGCCTGCC	456
	CTTTTCTGAA GAGAAGTGGT GGGAGAGTGG ATGGGGTGGG GGCAGAGGAA AAGTGGGGGA	516
	GAGGCCTGG GAGGAGAGGA GGGAGGGGGA CGGGGTGGGG GTGGGGAAAA CTATGGTTGG	576

GATGTAAAAA CGGATAATAA TATAAATATT AAATAAAAAG AGAGTATTGA GCA

629

5 (2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 amino acids

(B) TYPE: amino acid

10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn Asn Ser
 1 5 10 15

20 Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln Thr Val Phe Leu
 20 25 30

(2) INFORMATION FOR SEQ ID NO:27:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 379 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..69

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

48 TGC TTT GCC CCA AGA TGC AGA GAG AGA AGG AGG AAT GAG AGA TTG AGA
 Cys Phe Ala Pro Arg Cys Arg Glu Arg Arg Arg Asn Glu Arg Leu Arg
 1 5 10 15

45 AGG GAA AGT GTA CGC CCT GTA TAACAGTGTC CGCAGAAGCA AGGGGCTGAA 99
 Arg Glu Ser Val Arg Pro Val
 20

50 AAGATCTGAA GGTAGCCTCC GTCATCTCTT CTGGGATACA TGGATCGTGG GGATCATGAG 159

GCATTCTTCC CTTAACAAAT TTAAGCTGTT TTACCCACTA CCTCACCTTC TTAAAAACCT 219

CTTTCAGATT AAGCTGAACA GTTACAAGAT GGCTGGCATC CCTCTCCTTT CTCCCCATAT 279

55 GCAATTTGCT TAATGTAACC TCTTCTTTTG CCATGTTTCC ATTCTGCCAT CTTGAATTGT 339

CTTGTCAGCC AATTCATTAT CTATTAAACA CTAATTTGAG 379

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Phe Ala Pro Arg Cys Arg Glu Arg Arg Arg Asn Glu Arg Leu Arg
 1 5 10 15
 15 Arg Glu Ser Val Arg Pro Val
 20

(2) INFORMATION FOR SEQ ID NO:29:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 261 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- 30 (A) NAME/KEY: CDS
(B) LOCATION: 1..135

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

35 CAC AAG AAG CCG AAT CAG CCT AGC AGG CCC AGC AAC ACA GCC TCT AAG 48
 His Lys Lys Pro Asn Gln Pro Ser Arg Pro Ser Asn Thr Ala Ser Lys
 1 5 10 15
 40 TTA GAG CGG GAT AGT AAC GCT GAC AGA GAG ACT ATC AAC CTG AAG GAA 96
 Leu Glu Arg Asp Ser Asn Ala Asp Arg Glu Thr Ile Asn Leu Lys Glu
 20 25 30
 45 CTT GAA CCC CAA ATT GCT TCA GCA AAA CCA AAT GCA GAG TGAAGGCAGT 145
 Leu Glu Pro Gln Ile Ala Ser Ala Lys Pro Asn Ala Glu
 35 40 45
 GAGAGCCTGA GGAAAGAGTT AAAAATTGCT TTGCCTGAAA TAAGAAGTGC AGAGTTTCTC 205
 50 AGAATTCAAA AATGTTCTCA GCTGATTGGA ATTCTACAGT TGAATAATTA AAGAAC 261

(2) INFORMATION FOR SEQ ID NO:30:

55 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

5 His Lys Lys Pro Asn Gln Pro Ser Arg Pro Ser Asn Thr Ala Ser Lys
 1 5 10 15
 Leu Glu Arg Asp Ser Asn Ala Asp Arg Glu Thr Ile Asn Leu Lys Glu
 20 25 30
 10 Leu Glu Pro Gln Ile Ala Ser Ala Lys Pro Asn Ala Glu
 35 40 45

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 210 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..183

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAA TGG AAG AAG AAG AAG CGG CCT CGC AAC TCT TAT AAA TGT GGA ACC 48
 Lys Trp Lys Lys Lys Lys Arg Pro Arg Asn Ser Tyr Lys Cys Gly Thr
 1 5 10 15
 35 AAC ACA ATG GAG AGG GAA GAG AGT GAA CAG ACC AAG AAA AGA GAA AAA 96
 Asn Thr Met Glu Arg Glu Glu Ser Glu Gln Thr Lys Lys Arg Glu Lys
 20 25 30
 40 ATC CAT ATA CCT GAA AGA TCT GAT GAA GCC CAG CGT GTT TTT AAA AGT 144
 Ile His Ile Pro Glu Arg Ser Asp Glu Ala Gln Arg Val Phe Lys Ser
 35 40 45
 45 TCG AAG ACA TCT TCA TGC GAC AAA AGT GAT ACA TGT TTT TAATTAAAGA 193
 Ser Lys Thr Ser Ser Cys Asp Lys Ser Asp Thr Cys Phe
 50 55 60
 GTAAAGCCCA AAAAAAA 210

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 61 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

5 Lys Trp Lys Lys Lys Lys Arg Pro Arg Asn Ser Tyr Lys Cys Gly Thr
 1 5 10 15
 Asn Thr Met Glu Arg Glu Glu Ser Glu Gln Thr Lys Lys Arg Glu Lys
 20 25 30
 10 Ile His Ile Pro Glu Arg Ser Asp Glu Ala Gln Arg Val Phe Lys Ser
 35 40 45
 Ser Lys Thr Ser Ser Cys Asp Lys Ser Asp Thr Cys Phe
 50 55 60

15 (2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 359 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

25 (ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 249..359

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GAGTTTATA CCTCAATAGA CTCTTACTAG TTTCTCTTTT TCAGGTTGTG AACTCAACC 60
 35 TTCAAAGACA CTCTGTTCCA TTTCTGTGGA CTAATAGGAT CATCTTTAGC ATCTGCCGGG 120
 TGGATGCCAT CCAGGCTTCT TTTTCTACAT CTCTGTTTCT CGATTTTGTG GAGCCTAGGA 180
 40 GGTGCCTAAG CTCCATTGGC TCTAGATTCC TGGCTTTCCC CATCATGTTC TCAAAGCAT 240
 CTGAAGCT ATG GCT TGC AAT TGT CAG TTG ATG CAG GAT ACA CCA CTC CTC 290
 Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu
 1 5 10
 45 AAG TTT CCA TGT CCA AGG CTC AAT CTT CTC TTT GTG CTG CTG ATT CGT 338
 Lys Phe Pro Cys Pro Arg Leu Asn Leu Leu Phe Val Leu Leu Ile Arg
 15 20 25 30
 50 CTT TCA CAA GTG TCT TCA GAT 359
 Leu Ser Gln Val Ser Ser Asp
 35

55 (2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu Lys Phe
 1 5 10 15
 Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
 20 25 30
 Gln Val Ser Ser Asp
 35

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 416 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 318..416

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CCAAAGAAAA AGTGATTGT CATTGCTTTA TAGACTGTAA GAAGAGAACA TCTCAGAAGT 60
 GGAGTCTTAC CCTGAAATCA AAGGATTAA AGAAAAAGTG GAATTTTCT TCAGCAAGCT 120
 GTGAAACTAA ATCCACAACC TTTGGAGACC CAGGAACACC CTCCAATCTC TGTGTGTTTT 180
 GTAAACATCA CTGGAGGGTC TTCTACGTGA GCAATTGGAT TGTCATCAGC CCTGCCTGTT 240
 TTGCACCTGG GAAGTGCCCT GGTCTTACTT GGGTCCAAAT TGTGGCTTT CACTTTTGAC 300
 CCTAAGCATC TGAAGCC ATG GGC CAC ACA CGG AGG CAG GGA ACA TCA CCA 350
 Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro
 1 5 10
 TCC AAG TGT CCA TAC CTG AAT TTC TTT CAG CTC TTG GTG CTG GCT GGT 398
 Ser Lys Cys Pro Tyr Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly
 15 20 25
 CTT TCT CAC TTC TGT TCA 416
 Leu Ser His Phe Cys Ser
 30

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr
1 5 10 15
Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys
20 25 30

Ser

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 113 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 99..113

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GGAGCAAGCA GACGCGTAAG AGTGGCTCCT GTAGGCAGCA CGGACTTGAA CAACCAGACT 60
CCTGTAGACG TGTTCAGAA CTTACGGAAG CACCCACG ATG GAC CCC AGA TGC 113
Met Asp Pro Arg Cys
1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Asp Pro Arg Cys
1 5

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 124 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

10 (A) NAME/KEY: CDS
(B) LOCATION: 107..124

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

15 CACAGGGTGA AAGCTTTGCT TCTCTGCTGC TGTAACAGGG ACTAGCACAG ACACACGGAT 60
GAGTGGGGTC ATTTCCAGAT ATTAGGTCAC AGCAGAAGCA GCCAAA ATG GAT CCC 115
Met Asp Pro
1
20 CAG TGC ACT 124
Gln Cys Thr
5

25

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Asp Pro Gln Cys Thr
1 5

40 (2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

45 (A) LENGTH: 195 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 148..195

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

AGGAGCCTTA GGAGGTACGG GGAGCTCGCA AATACTCCTT TTGGTTTATT CTTACCACCT 60

TGCTTCTGTG TTCCTTGGA ATGCTGCTGT GCTTATGCAT CTGGTCTCTT TTTGGAGCTA 120

CAGTGGACAG GCATTTGTGA CAGCACT ATG GGA CTG AGT AAC ATT CTC TTT 171

Met Gly Leu Ser Asn Ile Leu Phe

5

1

5

GTG ATG GCC TTC CTG CTC TCT GGT

195

Val Met Ala Phe Leu Leu Ser Gly

10

15

10

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met Gly Leu Ser Asn Ile Leu Phe Val Met Ala Phe Leu Leu Ser Gly

1

5

10

15

25

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

40 CCAACATAAC TGAGTCTGGA AA

22

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

45

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

55 CTGGATTCTG ACTCACCTTC A

21

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

AGGTTAAGAG TGGTAGAGCC A

21

10

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

AATACCATGT ATCCACATG G

21

25

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

CTGAAGCTAT GGCTTGCAAT T

21

40

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

45

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

TGGCTTCTCT TTCCTTACCT T

21

(2) INFORMATION FOR SEQ ID NO: 49:

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

5

GCAAATGGTA GATGAGACTG T

21

(2) INFORMATION FOR SEQ ID NO: 50:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

20

CAACCGAGAA ATCTACCACT AA

22

(2) INFORMATION FOR SEQ ID NO: 51:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

35

GCCGGTAACA AGTCTCTTCA

20

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

40

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

AAAAGCTCTA TAGCATTCTG TC

22

50

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

55

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

ACTGACTTGG ACAGTTGTTC A

21

5 (2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

TTTGATGGAC AACTTTACTA

20

(2) INFORMATION FOR SEQ ID NO: 55:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

25 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

30

CAGCTCACTC AGGCTTATGT

20

(2) INFORMATION FOR SEQ ID NO: 56:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

45

AAACAGCATC TGAGATCAGC A

21

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

CTGAGATCAG CAAGACTGTC

20

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

CTGAAGCTAT GGCTTGCAAT T

21

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

ACAAGTGTCT TCAGATGTTG AT

22

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

CTGGATTCTG ACTCACCTTC A

21

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

CCAGGTGAAG TCCTCTGACA

20

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1417 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 249..884

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

5	GAGTTTTATA CCTCAATAGA CTCTTACTAG TTTCTCTTTT TCAGGTTGTG AAACCTCAACC	60
10	TTCAAAGACA CTCTGTTCCA TTTCTGTGGA CTAATAGGAT CATCTTTAGC ATCTGCCGGG	120
20	TGGATGCCAT CCAGGCTTCT TTTTCTACAT CTCTGTTTCT CGATTTTGTG GAGCCTAGGA	180
	GGTGCCTAAG CTCCATTGGC TCTAGATTCC TGGCTTTCCC CATCATGTTC TCCAAAGCAT	240
25	CTGAAGCT ATG GCT TGC AAT TGT CAG TTG ATG CAG GAT ACA CCA CTC CTC	290
	Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu	
	1 5 10	
30	AAG TTT CCA TGT CCA AGG CTC AAT CTT CTC TTT GTG CTG CTG ATT CGT	338
	Lys Phe Pro Cys Pro Arg Leu Asn Leu Leu Phe Val Leu Leu Ile Arg	
	15 20 25 30	
35	CTT TCA CAA GTG TCT TCA GAT GTT GAT GAA CAA CTG TCC AAG TCA GTG	386
	Leu Ser Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val	
	35 40 45	
40	AAA GAT AAG GTA TTG CTG CCT TGC CGT TAC AAC TCT CCT CAT GAA GAT	434
	Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp	
	50 55 60	
45	GAG TCT GAA GAC CGA ATC TAC TGG CAA AAA CAT GAC AAA GTG GTG CTG	482
	Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu	
	65 70 75	
50	TCT GTC ATT GCT GGG AAA CTA AAA GTG TGG CCC GAG TAT AAG AAC CGG	530
	Ser Val Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg	
	80 85 90	
55	ACT TTA TAT GAC AAC ACT ACC TAC TCT CTT ATC ATC CTG GGC CTG GTC	578
	Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val	
	95 100 105 110	
60	CTT TCA GAC CGG GGC ACA TAC AGC TGT GTC GTT CAA AAG AAG GAA AGA	626
	Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg	
	115 120 125	
65	GGA ACG TAT GAA GTT AAA CAC TTG GCT TTA GTA AAG TTG TCC ATC AAA	674
	Gly Thr Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys	
	130 135 140	

CCC CCA GAA GAC CCT CCT GAT AGC AAG AAC ACA CTT GTG CTC TTT GGG 722
 Pro Pro Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly
 145 150 155
 5 GCA GGA TTC GGC GCA GTA ATA ACA GTC GTC GTC ATC GTT GTC ATC ATC 770
 Ala Gly Phe Gly Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile
 160 165 170
 10 AAA TGC TTC TGT AAG CAC AGA AGC TGT TTC AGA AGA AAT GAG GCA AGC 818
 Lys Cys Phe Cys Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser
 175 180 185 190
 15 AGA GAA ACA AAC AAC AGC CTT ACC TTC GGG CCT GAA GAA GCA TTA GCT 866
 Arg Glu Thr Asn Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala
 195 200 205
 20 GAA CAG ACC GTC TTC CTT TAGTTCTTCT CTGTCCATGT GGGATACATG GTATTATGTG 924
 Glu Gln Thr Val Phe Leu
 210
 GCTCATGAGG TACAATCTTT CTTTCAGCAC CGTGCTAGCT GATCTTTCGG ACAACTTGAC 984
 ACAAGATAGA GTTAACTGGG AAGAGAAAGC CTTGAATGAG GATTTCCTTC CATCAGGAAG 1044
 25 CTACGGGCAA GTTTGCTGGG CCTTTGATTG CTTGATGACT GAAGTGGAAG GGCTGAGCCC 1104
 ACTGTGGGTG GTGCTAGCCC TGGGCAGGGG CAGGTGACCC TGGGTGGTAT AAGAAAAAGA 1164
 30 GCTGTCACTA AAAGGAGAGG TGCCTAGTCT TACTGCAACT TGATATGTCA TGTTTGTTG 1224
 GTGTCTGTGG GAGGCCTGCC CTTTTCTGAA GAGAAGTGGT GGGAGAGTGG ATGGGGTGGG 1284
 GGCAGAGGAA AAGTGGGGGA GAGGGCCTGG GAGGAGAGGA GGGAGGGGGA CGGGGTGGGG 1344
 35 GTGGGGAAAA CTATGTTTGG GATGTAAAAA CGGATAATAA TATAAATATT AAATAAAAAG 1404
 AGAGTATTGA GCA 1417

40

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 212 amino acids
 45 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu Lys Phe
 1 5 10 15
 55 Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
 20 25 30
 Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp
 35 40 45

Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser
 50 55 60
 5 Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu Ser Val
 65 70 75 80
 Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu
 85 90 95
 10 Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser
 100 105 110
 15 Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr
 115 120 125
 Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys Pro Pro
 130 135 140
 20 Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly
 145 150 155 160
 Phe Gly Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys
 165 170 175
 25 Phe Cys Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu
 180 185 190
 30 Thr Asn Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln
 195 200 205
 Thr Val Phe Leu
 210

35

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1606 base pairs
 40 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 249..926

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GAGTTTTATA CCTCAATAGA CTCTTACTAG TTTCTCTTTT TCAGGTTGTG AAACCTCAACC 60
 55 TTCAAAGACA CTCTGTTCCA TTTCTGTGGA CTAATAGGAT CATCTTTAGC ATCTGCCGGG 120
 TGGATGCCAT CCAGGCTTCT TTTTCTACAT CTCTGTTTCT CGATTTTTGT GAGCCTAGGA 180
 GGTGCCTAAG CTCCATTGGC TCTAGATTCC TGGCTTTCCC CATCATGTTC TCCAAAGCAT 240

	CTGAAGCT ATG GCT TGC AAT TGT CAG TTG ATG CAG GAT ACA CCA CTC CTC	290
	Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu	
	1 5 10	
5	AAG TTT CCA TGT CCA AGG CTC AAT CTT CTC TTT GTG CTG CTG ATT CGT	338
	Lys Phe Pro Cys Pro Arg Leu Asn Leu Leu Phe Val Leu Leu Ile Arg	
	15 20 25 30	
10	CTT TCA CAA GTG TCT TCA GAT GTT GAT GAA CAA CTG TCC AAG TCA GTG	386
	Leu Ser Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val	
	35 40 45	
15	AAA GAT AAG GTA TTG CTG CCT TGC CGT TAC AAC TCT CCT CAT GAA GAT	434
	Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp	
	50 55 60	
20	GAG TCT GAA GAC CGA ATC TAC TGG CAA AAA CAT GAC AAA GTG GTG CTG	482
	Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu	
	65 70 75	
25	TCT GTC ATT GCT GGG AAA CTA AAA GTG TGG CCC GAG TAT AAG AAC CGG	530
	Ser Val Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg	
	80 85 90	
30	ACT TTA TAT GAC AAC ACT ACC TAC TCT CTT ATC ATC CTG GGC CTG GTC	578
	Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val	
	95 100 105 110	
35	CTT TCA GAC CGG GGC ACA TAC AGC TGT GTC GTT CAA AAG AAG GAA AGA	626
	Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg	
	115 120 125	
40	GGA ACG TAT GAA GTT AAA CAC TTG GCT TTA GTA AAG TTG TCC ATC AAA	674
	Gly Thr Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys	
	130 135 140	
45	CCC CCA GAA GAC CCT CCT GAT AGC AAG AAC ACA CTT GTG CTC TTT GGG	722
	Pro Pro Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly	
	145 150 155	
50	GCA GGA TTC GGC GCA GTA ATA ACA GTC GTC GTC ATC GTT GTC ATC ATC	770
	Ala Gly Phe Gly Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile	
	160 165 170	
55	AAA TGC TTC TGT AAG CAC GGT CTC ATC TAC CAT TTG CAA CTG ACC TCT	818
	Lys Cys Phe Cys Lys His Gly Leu Ile Tyr His Leu Gln Leu Thr Ser	
	175 180 185 190	
60	TCT GCA AAG GAC TTC AGA AAC CTA GCA CTA CCC TGG CTC TGC AAA CAC	866
	Ser Ala Lys Asp Phe Arg Asn Leu Ala Leu Pro Trp Leu Cys Lys His	
	195 200 205	
65	GGT TCT CTA GGT GAA GCC TCT GCA GTG ATT TGC AGA AGT ACT CAG ACG	914
	Gly Ser Leu Gly Glu Ala Ser Ala Val Ile Cys Arg Ser Thr Gln Thr	
	210 215 220	

AAT GAA CCA CAG TAGTTCTGCT GTTTCTGAGG ACGTAGTTTA GAGACTGAAT 966
 Asn Glu Pro Gln
 225

5 TCTTTGGAAA GGACATAGGG ACAGTTTGCA CATTTGCTTG CACATCACAC ACACACACAC 1026
 ACACACACAC ACACACACAC ACACACACAC ACACACACAC ACACACACAC TCTCTCTCTC 1086
 TCTCTCTCTC GATACCTTAG GATAGGGTTC TACCCTGTTG CTCAGTGACA AAGAATCACT 1146
 10 CTGTGGCGGA GGCAGGCTTC AAGCTTGCAG CAATCCTCCT GCACCAGTTT CCTGAGTGCC 1206
 AGACTTCCAG GTGTAAGCTA TGGCACTTAG CAGAACACTA GCTGAATCAA TGAAGACACT 1266
 15 GAGGTTCCAA GAGGGAACCT GAATTATGAA GGTGAGTCAG AATCCAGATT TCCTGGCTCT 1326
 ACCACTCTTA ACCTGTATCT GTTAGACCCC AAGCTCTGAG CTCATAGACA AGCTAATTTA 1386
 AAATGCTTTT TAATAAGCAG AAGGCTCAGT TAGTACGGGG TTCAGGATAC TGCTTACTGG 1446
 20 CAATATTTGA CTAGCCTCTA TTTTGTGTGT TTTTAAAGG CCTACTGACT GTAGTGTAAT 1506
 TTGTAGGAAA CATGTTGCTA TGTATACCCA TTTGAGGGTA ATAAAAATGT TGGTAATTTT 1566
 25 CAGCCAGCAC TTTCCAGGTA TTTCCCTTTT TATCCTTCAT 1606

(2) INFORMATION FOR SEQ ID NO:65:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 226 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

40 Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu Lys Phe
 1 5 10 15
 Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
 20 25 30
 45 Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp
 35 40 45
 Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser
 50 55 60
 Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu Ser Val
 65 70 75 80
 55 Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu
 85 90 95
 Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser
 100 105 110

Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly
145 150 155 160

Phe Cys Lys His Gly Leu Ile Tyr His Leu Gln Leu Thr Ser Ser Ala
180 185 190

```

      Leu Gly Glu Ala Ser Ala Val Ile Cys Arg Ser Thr Gln Thr Asn Glu
20          210                      215                    220

```

Pro Gln
225

CLAIMS

1. An isolated nucleic acid encoding a protein which binds CD28 or CTLA4 comprising a contiguous nucleotide sequence derived from at least one T cell costimulatory molecule gene, the nucleotide sequence represented by a formula A-B-C-D-E, wherein

A comprises a nucleotide sequence of at least one first exon of a T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

10 B comprises a nucleotide sequence of at least one second exon of a T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin variable region-like domain,

15 C comprises a nucleotide sequence of at least one third exon of a T cell costimulatory molecule gene, wherein the at least one third exon encodes an immunoglobulin constant region-like domain,

D comprises a nucleotide sequence of at least one fourth exon of a T cell costimulatory molecule gene, wherein the at least one fourth exon encodes a transmembrane domain, and

20 E comprises a nucleotide sequence of at least one fifth exon of a T cell costimulatory molecule gene, wherein the at least one fifth exon encodes a cytoplasmic domain,

with the proviso that E does not comprise a nucleotide sequence selected from a group consisting of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29 and SEQ ID NO:31.

25

2. The isolated nucleic acid of claim 1 which is a cDNA.

3. The isolated nucleic acid of claim 2 which comprises a coding region of the cDNA.

30

4. The isolated nucleic acid of claim 1, wherein the nucleotide sequence is derived from a T cell costimulatory molecule gene encoding B7-1.

5. The isolated nucleic acid of claim 4, wherein B7-1 is murine.

35

6. The isolated nucleic acid of claim 4, wherein B7-1 is human.

7. The isolated nucleic acid of claim 5, wherein E comprises a nucleotide sequence shown in SEQ ID NO:4.

8. The isolated nucleic acid of claim 5, wherein E comprises a nucleotide sequence encoding an amino acid sequence shown in SEQ ID NO:5.

5 9. An isolated nucleic acid encoding a protein which binds CD28 or CTLA4 and is encoded by a T cell costimulatory molecule gene having

at least one first exon encoding a first cytoplasmic domain comprising a nucleotide sequence selected from the group consisting of a nucleotide sequence of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29 and SEQ ID NO:31, and

10 at least one second exon encoding a second cytoplasmic domain, wherein the isolated nucleic acid comprises a nucleotide sequence encoding the second cytoplasmic domain.

15 10. The isolated nucleic acid of claim 9 which comprises a coding region of a cDNA.

11. The isolated nucleic acid of claim 9 which does not comprise a nucleotide sequence encoding the first cytoplasmic domain.

20 12. The isolated nucleic acid of claim 9 wherein the T cell costimulatory molecule gene is B7-1.

13. The isolated nucleic acid of claim 12 wherein B7-1 is murine.

25 14. The isolated nucleic acid of claim 12 wherein B7-1 is human.

15. An isolated nucleic acid encoding a protein which binds CD28 or CTLA4 comprising a nucleotide sequence shown in SEQ ID NO:1.

30 16. An isolated nucleic acid encoding a protein which binds CD28 or CTLA4 comprising a nucleotide sequence shown in SEQ ID NO:3.

35 17. An isolated nucleic acid encoding a cytoplasmic domain derived from a protein which binds CD28 or CTLA4, the nucleic acid comprising a nucleotide sequence shown in SEQ ID NO:4.

18. An isolated protein which binds to CD28 or CTLA4 having an amino acid sequence derived from amino acid sequences encoded by at least one T cell costimulatory molecule gene, the protein comprising a contiguous amino acid sequence represented by a formula A-B-C-D-E, wherein

5

A, which may or may not be present, comprises an amino acid sequence of a signal peptide domain encoded by at least one exon of a T cell costimulatory molecule gene,

10

B comprises an amino acid sequence of an immunoglobulin variable region-like domain encoded by at least one exon of a T cell costimulatory molecule gene,

C comprises an amino acid sequence of an immunoglobulin constant region-like domain encoded by at least one exon of a T cell costimulatory molecule gene,

D comprises an amino acid sequence of a transmembrane domain encoded by at least one exon of a T cell costimulatory molecule gene, and

15

E comprises an amino acid sequence of a cytoplasmic domain encoded by at least one exon of a T cell costimulatory molecule gene,

with the proviso that E not comprise an amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30 and SEQ ID NO:32.

20

19. The isolated protein of claim 18 which is B7-1.

20. The isolated protein of claim 19 which is murine.

25

21. The isolated protein of claim 19 which is human.

22. The isolated protein of claim 20, wherein E comprises an amino acid sequence shown in SEQ ID NO:5.

30

23. An isolated protein which binds CD28 or CTLA4 and is encoded by a T cell costimulatory molecule gene having

at least one first exon encoding a first cytoplasmic domain comprising an amino acid sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, and SEQ ID NO:32, and

35

at least one second exon encoding a second cytoplasmic domain, wherein the T cell costimulatory molecule comprises the second cytoplasmic domain.

24. The isolated protein of claim 23 which does not comprise the first cytoplasmic domain.

25. The isolated protein of claim 23 which is B7-1.

26. The isolated protein of claim 25 which is murine.

27. The isolated protein of claim 25 which is human.

28. An isolated protein which binds CD28 or CTLA4 comprising an amino acid sequence shown in SEQ ID NO:2.

29. An isolated cytoplasmic domain polypeptide derived from a protein which binds CD28 or CTLA4, the polypeptide comprising an amino acid sequence shown in SEQ ID NO:5.

30. A recombinant expression vector comprising the nucleic acid molecule of claim 15.

31. A host cell which contains the recombinant expression vector of claim 30.

32. An antibody which binds to the murine B7-1 cytoplasmic domain polypeptide of claim 29.

33. An isolated nucleic acid encoding a protein which binds CD28 or CTLA4 comprising a contiguous nucleotide sequence derived from at least one T cell costimulatory molecule gene, the nucleotide sequence represented by a formula A-B-C-D-E, wherein

A comprises a nucleotide sequence of at least one first exon of a T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

B comprises a nucleotide sequence of at least one second exon of a T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin variable region-like domain,

C comprises a nucleotide sequence of at least one third exon of a T cell costimulatory molecule gene, wherein the at least one third exon encodes an immunoglobulin constant region-like domain,

D, which may or may not be present, comprises a nucleotide sequence of at least one fourth exon of a T cell costimulatory molecule gene, wherein the at least one fourth exon encodes a transmembrane domain, and

E, which may or may not be present, comprises a nucleotide sequence of at least one fifth exon of a T cell costimulatory molecule gene, wherein the at least one fifth exon encodes a cytoplasmic domain,

5 with the proviso that A does not comprise a nucleotide sequence selected from a group consisting of SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39 and SEQ ID NO:41.

10 34. The isolated nucleic acid of claim 33 which is a cDNA.

35. The isolated nucleic acid of claim 34 which comprises a coding region of the cDNA.

15 36. The isolated nucleic acid of claim 33, wherein the nucleotide sequence is derived from a T cell costimulatory molecule gene encoding B7-2.

37. The isolated nucleic acid of claim 36, wherein B7-2 is murine.

20 38. The isolated nucleic acid of claim 36, wherein B7-2 is human.

39. The isolated nucleic acid of claim 37, wherein A comprises a nucleotide sequence shown in SEQ ID NO:14.

25 40. An isolated nucleic acid encoding a protein which binds CD28 or CTLA4 and is encoded by a T cell costimulatory molecule gene having

at least one first exon encoding a first signal peptide domain comprising a nucleotide sequence selected from the group consisting of a nucleotide sequence of SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37 SEQ ID NO:39 and SEQ ID NO:41, and

30 at least one second exon encoding a second signal peptide domain, wherein the isolated nucleic acid comprises a nucleotide sequence encoding the second signal peptide domain.

35 41. The isolated nucleic acid of claim 40 which comprises a coding region of a cDNA.

42. The isolated nucleic acid of claim 40 which does not comprise a nucleotide sequence encoding the first signal peptide domain.

43. The isolated nucleic acid of claim 40 wherein the T cell costimulatory molecule gene is B7-2.

44. The isolated nucleic acid of claim 43 wherein B7-2 is murine.

45. The isolated nucleic acid of claim 43 wherein B7-2 is human.

46. An isolated nucleic acid encoding a protein which binds CD28 or CTLA4 comprising a nucleotide sequence shown in SEQ ID NO:12.

47. An isolated nucleic acid encoding a signal peptide domain derived from a protein which binds CD28 or CTLA4, the nucleic acid comprising a nucleotide sequence shown in SEQ ID NO:14.

48. An isolated protein which binds CD28 or CTLA4 having an amino acid sequence derived from amino acid sequences encoded by at least one T cell costimulatory molecule gene, the protein comprising a contiguous amino acid sequence represented by a formula A-B-C-D-E, wherein

A comprises an amino acid sequence of a signal peptide domain encoded by at least one exon of a T cell costimulatory molecule gene,

B comprises an amino acid sequence of an immunoglobulin variable region-like domain encoded by at least one exon of a T cell costimulatory molecule gene,

C comprises an amino acid sequence of an immunoglobulin constant region-like domain encoded by at least one exon of a T cell costimulatory molecule gene,

D, which may or may not be present, comprises an amino acid sequence of a transmembrane domain encoded by at least one exon of a T cell costimulatory molecule gene, and

E, which may or may not be present, comprises an amino acid sequence of a cytoplasmic domain encoded by at least one exon of a T cell costimulatory molecule gene,

with the proviso that A not comprise an amino acid sequence selected from the group consisting of SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40 and SEQ ID NO: 42.

49. The isolated protein of claim 48 which is B7-2.

50. The isolated protein of claim 49 which is murine.

51. The isolated protein of claim 49 which is human.

52. The isolated protein of claim 50, wherein A comprises an amino acid sequence
5 shown in SEQ ID NO: 15.

53. An isolated protein which binds CD28 or CTLA4 and is encoded by a T cell costimulatory molecule gene having

at least one first exon encoding a first signal peptide domain comprising an amino
10 acid sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40 and SEQ ID NO:42, and

at least one second exon encoding a second signal peptide domain,
wherein the T cell costimulatory molecule comprises the second signal peptide domain.

15 54. The isolated protein of claim 53 which does not comprise the first signal peptide domain.

55. The isolated protein of claim 53 which is B7-2.

20 56. The isolated protein of claim 55 which is murine.

57. The isolated protein of claim 55 which is human.

58. An isolated protein which binds CD28 or CTLA4 comprising an amino acid
25 sequence shown in SEQ ID NO:13.

59. An isolated signal peptide domain polypeptide derived from a protein which binds CD28 or CTLA4, the polypeptide comprising an amino acid sequence shown in SEQ ID NO:15.

30

60. A recombinant expression vector comprising the nucleic acid molecule of claim 46.

61. A host cell which contains the recombinant expression vector of claim 60.

35

62. An antibody which binds to the polypeptide of claim 59.

63. An isolated nucleic acid encoding a protein comprising a contiguous nucleotide sequence derived from at least one T cell costimulatory molecule gene, the nucleotide sequence represented by a formula A-B-C-D, wherein

5 A comprises a nucleotide sequence of at least one first exon of a T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

 B comprises a nucleotide sequence of at least one second exon of a T cell costimulatory molecule gene, wherein the at least one second exon encodes an
10 immunoglobulin constant region-like domain,

 C comprises a nucleotide sequence of at least one third exon of a T cell costimulatory molecule gene, wherein the at least one third exon encodes a transmembrane domain, and

 D comprises a nucleotide sequence of at least one fourth exon of a T cell
15 costimulatory molecule gene, wherein the at least one fourth exon encodes a cytoplasmic domain.

64. The isolated nucleic acid of claim 63 comprising a nucleotide sequence shown
20 in SEQ ID NO:8.

65. The isolated nucleic acid of claim 63 comprising a nucleotide sequence shown
in SEQ ID NO:10.

66. An isolated protein having an amino acid sequence derived from amino acid
25 sequences encoded by at least one T cell costimulatory molecule gene, the protein comprising a contiguous amino acid sequence represented by a formula A-B-C-D, wherein

 A, which may or may not be present, comprises an amino acid sequence of a
30 signal peptide domain encoded by at least one exon of a T cell costimulatory molecule gene,

 B comprises an amino acid sequence of an immunoglobulin constant region-like domain encoded by at least one exon of a T cell costimulatory molecule gene, and

 C comprises an amino acid sequence of a transmembrane domain encoded by
at least one exon of a T cell costimulatory molecule gene, and

 D comprises an amino acid sequence of a cytoplasmic domain encoded by at
35 least one exon of a T cell costimulatory molecule gene.

67. The isolated protein of claim 66 comprising an amino acid sequence shown in
SEQ ID NO:9.

68. The isolated protein of claim 66 comprising an amino acid sequence shown in SEQ ID NO:11.

5 69. An isolated nucleic acid encoding a protein comprising a contiguous nucleotide sequence derived from at least one T cell costimulatory molecule gene, the nucleotide sequence represented by a formula A-B-C-D, wherein

10 A comprises a nucleotide sequence of at least one first exon of a T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

B comprises a nucleotide sequence of at least one second exon of a T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin variable region-like domain,

15 C comprises a nucleotide sequence of at least one third exon of a T cell costimulatory molecule gene, wherein the at least one third exon encodes a transmembrane domain, and

20 D comprises a nucleotide sequence of at least one fourth exon of a T cell costimulatory molecule gene, wherein the at least one fourth exon encodes a cytoplasmic domain.

70. The isolated nucleic acid of claim 69 comprising a nucleotide sequence shown in SEQ ID NO:62.

25 71. The isolated nucleic acid of claim 69 comprising a nucleotide sequence shown in SEQ ID NO:64.

30 72. An isolated protein having an amino acid sequence derived from amino acid sequences encoded by at least one T cell costimulatory molecule gene, the protein comprising a contiguous amino acid sequence represented by a formula A-B-C-D, wherein

A, which may or may not be present, comprises an amino acid sequence of a signal peptide domain encoded by at least one exon of a T cell costimulatory molecule gene,

35 B comprises an amino acid sequence of an immunoglobulin variable region-like domain encoded by at least one exon of a T cell costimulatory molecule gene, and

C comprises an amino acid sequence of a transmembrane domain encoded by at least one exon of a T cell costimulatory molecule gene, and

D comprises an amino acid sequence of a cytoplasmic domain encoded by at least one exon of a T cell costimulatory molecule gene.

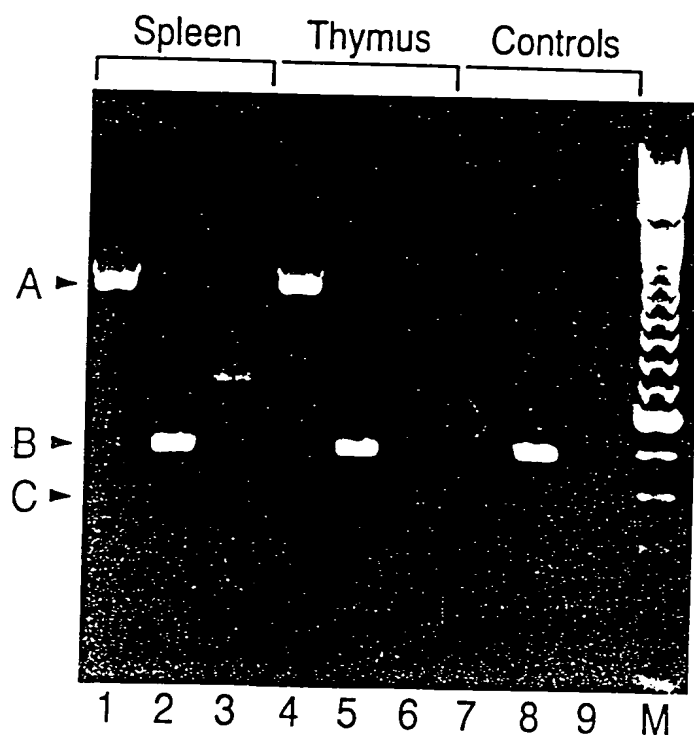
73. The isolated protein of claim 72 comprising an amino acid sequence shown in
5 SEQ ID NO:63.

74. The isolated protein of claim 72 comprising an amino acid sequence shown in
SEQ ID NO:65.

10 75. A recombinant expression vector comprising the nucleic acid molecule of
claim 69.

76. A host cell which contains the recombinant expression vector of claim 75.



**FIGURE 1**

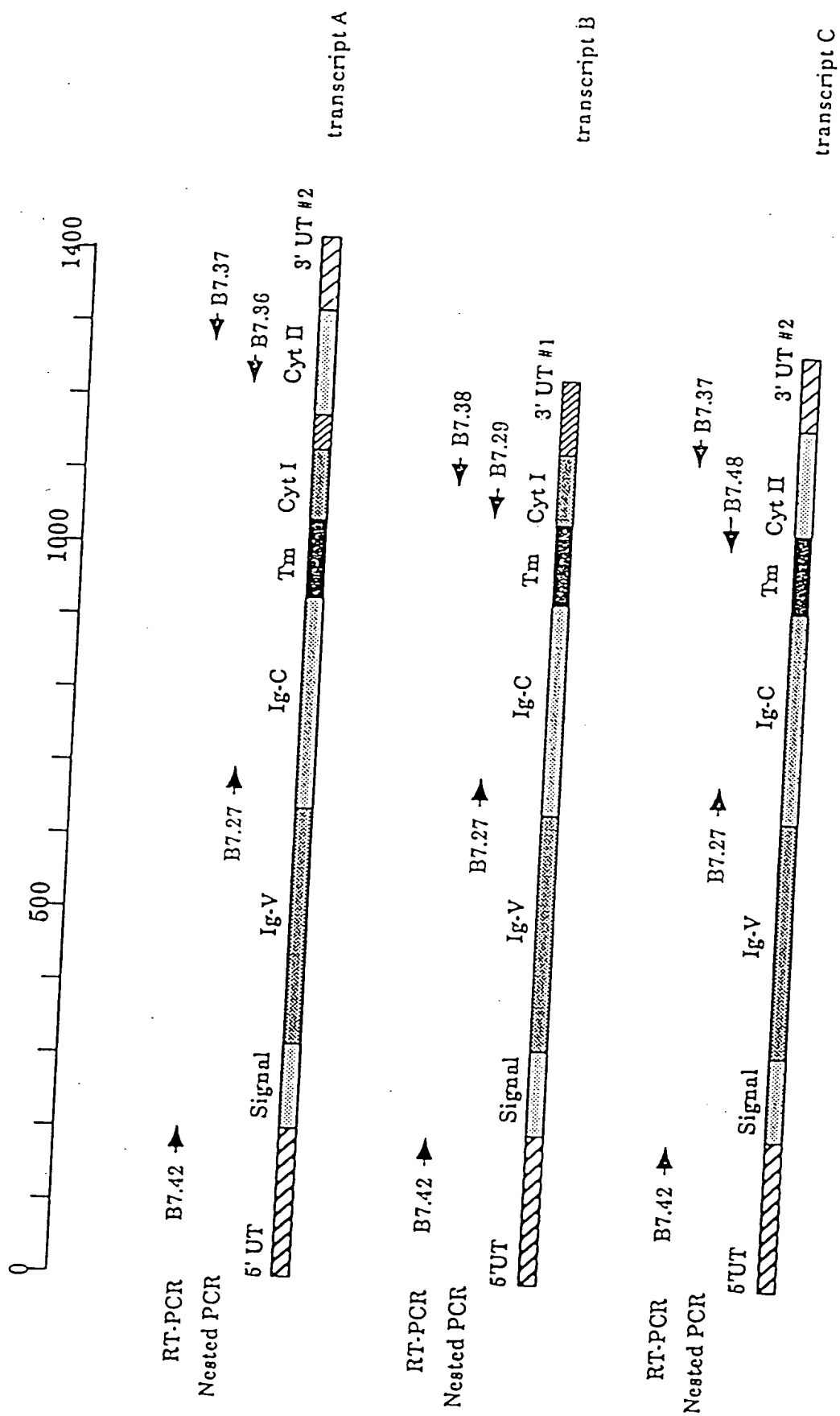


FIGURE 2

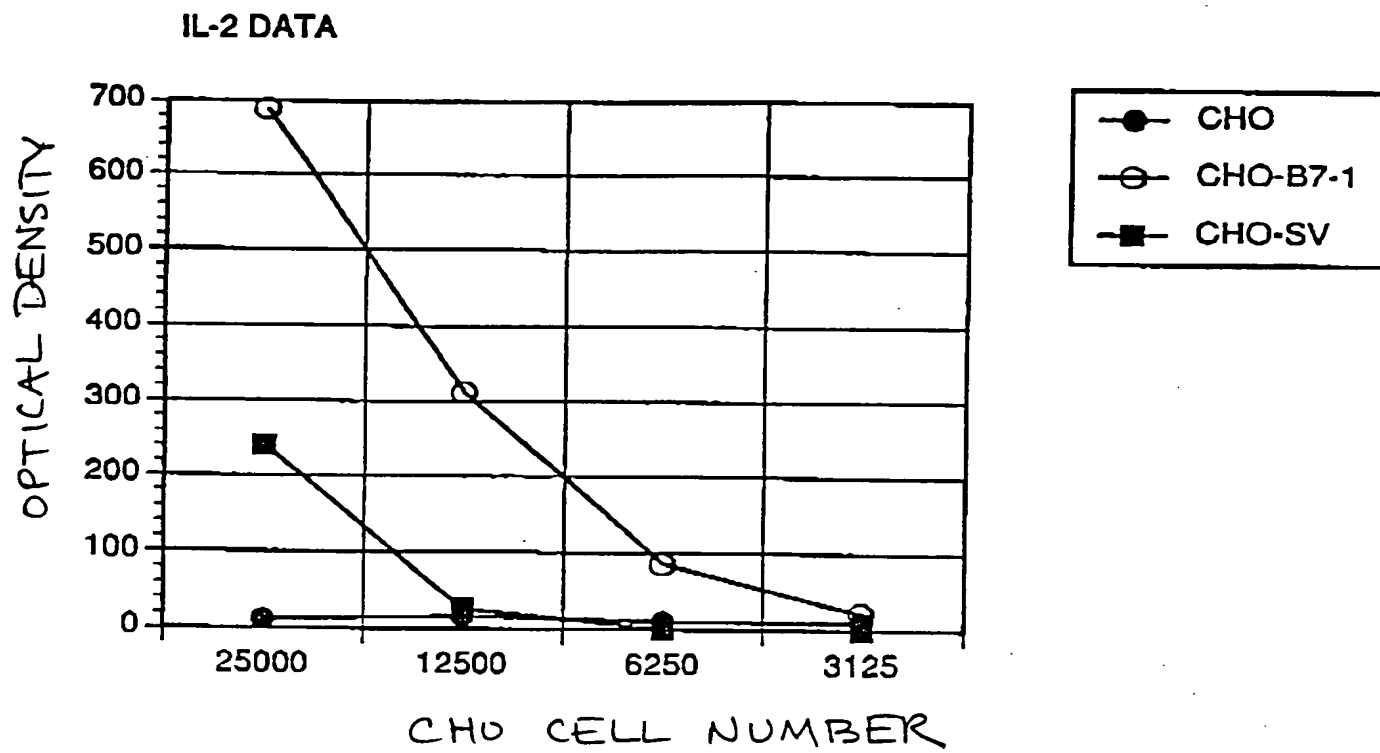
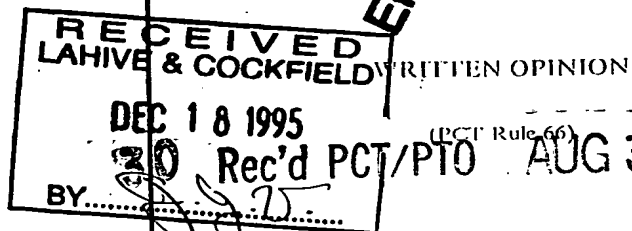


FIGURE 3

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

MANDRAGOURAS, Amy E.
Lahive & Cockfield
60 State Street
Boston, MA 02109
ETATS-UNIS D'AMERIQUE



Date of mailing
(day/month/year)

18 DEC 1995

Applicant's or agent's file reference

BWI-120CPPC

REPLY DUE

within 3 months/days
from the above date of mailing

International application No.

PCT/US 95/02576

International filing date (day/month/year)

02/03/1995

Priority date (day/month/year)

02/03/1994

International Patent Classification (IPC) or both national classification and IPC:

C12N15/12

Applicant

BRIGHAM AND WOMEN'S HOSPITAL et al.

1. This written opinion is the first (first, etc.) drawn up by this International Preliminary Examining Authority.

2. This report contains indications and corresponding pages relating to the following items:

- I ☒ Basis of the opinion
 II ☐ Priority
 III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 IV ☐ Lack of unity of invention
 V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 VI ☐ Certain documents cited
 VII ☐ Certain defects in the international application
 VIII ☒ Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
 For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4bis.
 For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 02/07/1996

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu-d
Fax: (+49-89) 2399-4465

Authorized officer

Examiner

K. Giebler

Formalities officer
(incl. extension of time limits)
Telephone No.

Peter Ehrenreich

WRITTEN OPINION

Intern. application No.

PCT/US95/02576

I. Basis of the opinion

1. This opinion has been drawn up on the basis of (Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".):

☒ the international application as originally filed.

☐ the description, pages _____, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,

☐ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. _____, filed with the letter of _____,

☐ the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____.
☐ the claims, Nos. _____.
☐ the drawings, sheets/fig _____.

3. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

WRITTEN OPINION

Intern. application No.
PCT/US95/02576

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)

Claims 31, 61, 63-64, 66-67, 69-76
Claims

Inventive Step (IS)

Claims 1-6, 9-14, 18-21, 23-27, 30, 33-38, 40-45, 48-51, 53-57
Claims

Industrial Applicability (IA)

Claims
Claims

2. CITATIONS AND EXPLANATIONS

1. The following documents are cited:

- D1 = J. EXP. MED. (1993) 178: 2185
- D2 = J. IMMUNOL. (1994) 153: 5038
- D3 = SCIENCE (1993) 262: 909
- D4 = NATURE (1993) 366: 76
- D5 = J. IMMUNOL. 143: 2718
- D6 = BIOCHEM. BIOPHYS. RES. COMM. (1994) 200: 443
- D7 = J. EXP. MED. (1991) 174: 625

2. The priority of the present application is only valid for the subject-matter of claims 1-68. Thus, the documents D2 and D6 constitute relevant prior art for claims 69-76.

3. The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 31, 61, 63-64, 66-67, and 69-76 is not new.

Claims 31, 61 and 76 lack novelty over naturally occurring mouse (or human) cells, which naturally contain and express the nucleic acids in question.

Claim 63 is not novel over known costimulatory molecule genes (D1, D3, D4, D5, D7), due to the wording "comprises" used for the definition of A, B, C, and D. The claim does not exclude that a sequence encoding a variable domain is "comprised" in "A" or "B". Also, the nucleic acid of claim 64 may "comprise" sequences in addition to those of SEQ ID NO:8. For the same reasons, claims 66-67, 69-70, 72-73, 75-76 also lack novelty over these documents, and claims 71 and 74 additionally lack novelty over D2. Furthermore, claims 69, 75 and 76 lack novelty over D6.

4. The present application does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of claims 1-6, 9-14, 18-21, 23-27, 30, 33-38, 40-45, 48-51, and 53-57 does not involve an inventive step (Rule 65(1)(2) PCT).

Claims 1-6 and 11 lack an inventive step since they include nucleic acids which differ from those known from D1, D3-D5 and D7 by only minor modifications (substitutions/additions/deletions/insertions), for example due to allelic variation. It was however obvious to a person skilled in the art that such variants could be obtained. Accordingly, claims 18-21, 23-27 and 30 also lack an inventive step.

Claim 9 does not involve an inventive step because it is completely unclear what the "second exon" should be. The claims even encompass the case where the "first exon" is identical to the "second exon". The same applies to claims 10 and 12-14.

Claims 33-38 and 42 lack an inventive step since they merely disclaim the five known signal sequences without defining the "first exon" by technical features. It was

obvious to a person skilled in the art that a certain degree of variation would exist in the signal sequence domain. Concerning claim 40, the person skilled in the art is not given any information about the nature of the signal peptide domain, and claims 40-41 and 43-45 consequently lack an inventive step. Claims 48-51 and 53-57 lack an inventive step for the same reasons.

5. However, claims 7, 8, 15-17, 22, 28-29, 32, 39, 46-47, 52, 58-60, 62, 65, and 68 which refer to specific sequences including the newly identified "exon 6" are considered to be both novel and inventive.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. The claims as a whole are not clear and concise, contrary to Article 6 PCT, because there are too many independent claims. There are 11 (!) independent claims directed to nucleic acids and 10 (!) independent claims directed to proteins. The claims should be recast to include only the minimum necessary number of independent claims in any one category, with dependent claims as appropriate (Rule 6.4(a)-(c) PCT).
2. The claims are furthermore unclear since the term "E" used in claim 1 is not sufficiently defined by technical features. The person skilled in the art is left completely in the dark about the nature of this sequence. The same applies to the nature of the "second cytoplasmic domain" and the "second signal peptide domain" which are referred to throughout the claims.
3. Claims 11, 24, 42, and 54 are particularly unclear since they are formulated as dependent claims, although from their content they are independent claims.



DANA-FARBER
CANCER INSTITUTE

44 Binney Street, Boston, MA 02115

THE JIMMY FUND

Certificate of Corporate Authority

Brian S. Meyer, Assistant Secretary of Dana-Farber Cancer Institute, Inc., a Massachusetts not-for-profit corporation with a usual place of business in Boston, Massachusetts, hereby certifies that at a meeting of the Executive Committee of its Board of Trustees duly held for the purpose in Boston on 17 January 1995, at which meeting a quorum was present and acting throughout, the following vote, upon motion duly made and seconded, was unanimously adopted.

VOTED: to amend and approve the following **CORPORATE AUTHORITIES:**

Commercially Sponsored Research Agreements - Director for Research, Director of Research Administration, President, singly.

Clinical Trial Agreements - Director for Research, Director of Research Administration, President, singly.

Copyright, Patent and Trademark Applications and Related Documents - Patent Counsel, Director, Office of Technology Transfer, General Counsel, Director for Research, singly.

Intellectual Property Management Agreements - Patent Counsel, Director, Office of Technology Transfer, General Counsel, Director for Research, singly.

Confidential Disclosure Agreements - Director, Office of Technology Transfer, Patent Counsel, Licensing Associate and Technology Manager, Office of Technology Transfer, singly.

Material Transfer Agreements - Director for Research, Director, Office of Technology Transfer, Director of Research Administration, Patent Counsel, General Counsel, singly.

Transaction Term Sheets - Director, Office of Technology Transfer, Licensing Associate and Technology Manager, Office of Technology Transfer, Director for Research, singly.


License and Option Agreements - Director for Research, Director of Research Administration, President, singly.

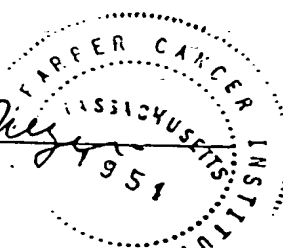
Documentation related to options for, and ownership of, equity or bonds in commercial entities as part of technology transfer transactions - Chief Operating Officer, Chief Financial Officer, Treasurer, Assistant Treasurers, Controller, singly.

Research Grant/Contract Applications (non-commercial sponsors) - Director for Research, Director of Research Administration, Director, Office of Grants and Contracts, President, singly.

Said Brian S. Meyer further certifies that he is the Assistant Secretary of Dana-Farber Cancer Institute, Inc. and that Prof. Christopher T. Walsh is the President, Bernard W. Janicki is the Director for Research, Julia Hart is the Patent Counsel, Kenneth P. Trevett is the General Counsel, Jeannette A. Potts is the Licensing Associate, Robert J. Distel is the Technology Manager, Dorothy E. Puhy is the Assistant Treasurer and Chief Financial Officer and that the aforesaid vote is in compliance with the Articles of Organization and Bylaws of said corporation, which have not been altered or amended, and are in full force and effect.

Executed this 22nd day of September 19 95


Brian S. Meyer, Assistant Secretary



From the RECEIVING OFFICE

PCT

To:

AMY E. MANDRAGOURAS
LAHIVE & COCKFIELD
60 STATE STREET
BOSTON, MA 02108

INTERED
Rec'd PCT/PTO
AUG 30 1996
8 Feb. 24, 1996 Response

COMMUNICATION IN CASES FOR WHICH
NO OTHER FORM IS APPLICABLE

Date of mailing
(day/month/year)

24 JAN 1996

Applicant's or agent's file reference
BWI-120CPPC

REPLY DUE

See paragraph 1 below

International application No.

PCT/US95/02576

International filing date
(day/month/year)

02 MAR 95

Applicant

BRIGHAM AND WOMEN'S HOSPITAL

- ☐ REPLY DUE within ONE MONTH from the above date of mailing
☐ NO REPLY DUE, however, see below _____
☒ IMPORTANT COMMUNICATION
☐ INFORMATION ONLY

2. COMMUNICATION:

As to the present international application, the power of attorney submitted 09 November 1995 for Dana-Farber Cancer Institute has been found insufficient in that the signature of JULIA D. HART, signing as Patent Counsel, does not infer authority to sign for Dana-Farber Cancer Institute. The general titles authorized are president, vice-president, secretary and treasurer. A resolution/copy of the by-laws authorizing signatory status is required.

RECEIVED
LAHIVE & COCKFIELD

JAN 30 1996

BY...

J. J. V.

Name and mailing address of the receiving Office
Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231

Attn: RO/US

Facsimile No.

Authorized officer

Sigfried Hostad
International Division RO/US
(703) 305-3680

Telephone No.

PCT


INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BWI-120CPPC	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US 95/ 02576	International filing date (day/month/year) 02/03/1995	Priority date (day/month/year) 02/03/1994
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant BRIGHAM AND WOMEN'S HOSPITAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
- ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).
- These annexes consists of a total of _____ sheets.

3. This report contains indications and corresponding pages relating to the following items:
- I ☒ Basis of the report
 - II ☐ Priority
 - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☒ Certain observations on the international application

Date of submission of the demand 28/09/1995	Date of completion of this report 16 APR 1996
Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer K. Giebler Telephone No.

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

☒ the international application as originally filed.

☐ the description, pages _____, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____,

☐ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. _____, filed with the letter of _____,
Nos. _____, filed with the letter of _____,

☐ the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____.
☐ the claims, Nos. _____.
☐ the drawings, sheets/fig _____.

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/US95/02576

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-30, 32-60, 62, 65, 68	YES
	Claims 31, 61, 63-64, 66-67, 69-76	NO
Inventive Step (IS)	Claims 7, 8, 15-17, 22, 28-29, 32, 39, 46-47, 52, 58-60, 62, 65, 68	YES
	Claims 1-6, 9-14, 18-21, 23-27, 30, 33-38, 40-45, 48-51, 53-57	NO
Industrial Applicability (IA)	Claims 1-76	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

1. The following documents are cited:

D1 = J. EXP. MED. (1993) 178: 2185

D2 = J. IMMUNOL. (1994) 153: 5038

D3 = SCIENCE (1993) 262: 909

D4 = NATURE (1993) 366: 76

D5 = J. IMMUNOL. 143: 2718

D6 = BIOCHEM. BIOPHYS. RES. COMM. (1994) 200: 443

D7 = J. EXP. MED. (1991) 174: 625

2. The priority of the present application is only valid for the subject-matter of claims 1-68. Thus, the documents D2 and D6 constitute relevant prior art for claims 69-76.

3. The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 31, 61, 63-64, 66-67, and 69-76 is not new.

Claims 31, 61 and 76 lack novelty over naturally occurring mouse (or human) cells, which naturally contain and express the nucleic acids in question.

Claim 63 is not novel over known costimulatory molecule genes (D1, D3, D4, D5, D7), due to the wording "comprises" used for the definition of A, B, C, and D. The claim does not exclude that a sequence encoding a variable domain is "comprised" in "A" or "B". Also, the nucleic acid of claim 64 may "comprise" sequences in addition to those of SEQ ID NO:8. For the same reasons, claims 66-67, 69-70, 72-73, 75-76 also lack novelty over these documents, and claims 71 and 74 additionally lack novelty over D2. Furthermore, claims 69, 75 and 76 lack novelty over D6.

4. The present application does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of claims 1-6, 9-14, 18-21, 23-27, 30, 33-38, 40-45, 48-51, and 53-57 does not involve an inventive step (Rule 65(1)(2) PCT).

Claims 1-6 and 11 lack an inventive step since they include nucleic acids which differ from those known from D1, D3-D5 and D7 by only minor modifications (substitutions/additions/deletions/insertions), for example due to allelic variation. It was however obvious to a person skilled in the art that such variants could be obtained. Accordingly, claims 18-21, 23-27 and 30 also lack an inventive step.

Claim 9 does not involve an inventive step because it is completely unclear what the "second exon" should be. The claims even encompass the case where the "first exon" is identical to the "second exon". The same applies to claims 10 and 12-14.

Claims 33-38 and 42 lack an inventive step since they merely disclaim the five known signal sequences without defining the "first exon" by technical features. It was

obvious to a person skilled in the art that a certain degree of variation would exist in the signal sequence domain. Concerning claim 40, the person skilled in the art is not given any information about the nature of the signal peptide domain, and claims 40-41 and 43-45 consequently lack an inventive step. Claims 48-51 and 53-57 lack an inventive step for the same reasons.

5. However, claims 7, 8, 15-17, 22, 28-29, 32, 39, 46-47, 52, 58-60, 62, 65, and 68 which refer to specific sequences including the newly identified "exon 6" are considered to be both novel and inventive.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. The claims as a whole are not clear and concise, contrary to Article 6 PCT, because there are too many independent claims. There are 11 independent claims directed to nucleic acids and 10 independent claims directed to proteins.
2. The claims are furthermore unclear since the term "E" used in claim 1 is not sufficiently defined by technical features. The person skilled in the art is left completely in the dark about the nature of this sequence. The same applies to the nature of the "second cytoplasmic domain" and the "second signal peptide domain" which are referred to throughout the claims.
3. Claims 11, 24, 42, and 54 are particularly unclear since they are formulated as dependent claims, although from their content they are independent claims.

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark
Office
(Box PCT)
Washington D.C. 20231
United States of America

in its capacity as elected Office

Date of mailing (day/month/year) 10 November 1995 (10.11.95)	
International application No. PCT/US95/02576	Applicant's or agent's file reference BWI-120CPPC
International filing date (day/month/year) 02 March 1995 (02.03.95)	Priority date (day/month/year) 02 March 1994 (02.03.94)
Applicant SHARPE, Arlene, H. et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

28 September 1995 (28.09.95)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Ellen Moyse

Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

PCT

RECEIVED 19 APR 1996



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 33 and Rule 70)

Applicant's or agent's file reference BWI-120CPPC	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPC/416)
International application No. PCT/US 95/ 02576	International filing date (day/month/year) 02/03/1995	Priority date (day/month/year) 02/03/1994
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant BRIGHAM AND WOMEN'S HOSPITAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
- ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).
- These annexes consists of a total of _____ sheets.

3. This report contains indications and corresponding pages relating to the following items:
- I ☒ Basis of the report
 - II ☐ Priority
 - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - .VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☒ Certain observations on the international application

Date of submission of the demand 28/09/1995	Date of completion of this report 16 APR 1996
Name and mailing address of the IPEA  European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer  K. Giebler Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/US95/02576

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

☒ the international application as originally filed.

☐ the description, pages _____, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____.

☐ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. _____, filed with the letter of _____,
Nos. _____, filed with the letter of _____.

☐ the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

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☐ the description, pages _____.
☐ the claims, Nos. _____.
☐ the drawings, sheets/fig _____.

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/US95/02576

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N) Claims 1-30, 32-60, 62, 65, 68 _____ YES

Claims 31, 61, 63-64, 66-67, 69-76 _____ NO

Inventive Step (IS) Claims 7, 8, 15-17, 22, 28-29, 32, 39, 46-47, 52, 58-60, 62, 65, 68 YES

Claims 1-6, 9-14, 18-21, 23-27, 30, 33-38, 40-45, 48-51, 53-57 NO

Industrial Applicability (IA) Claims 1-76 _____ YES

Claims _____ NO

2. CITATIONS AND EXPLANATIONS

1. The following documents are cited:

D1 = J. EXP. MED. (1993) 178: 2185

D2 = J. IMMUNOL. (1994) 153: 5038

D3 = SCIENCE (1993) 262: 909

D4 = NATURE (1993) 366: 76

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1. The claims as a whole are not clear and concise, contrary to Article 6 PCT, because there are too many independent claims. There are 11 independent claims directed to nucleic acids and 10 independent claims directed to proteins.
2. The claims are furthermore unclear since the term "E" used in claim 1 is not sufficiently defined by technical features. The person skilled in the art is left completely in the dark about the nature of this sequence. The same applies to the nature of the "second cytoplasmic domain" and the "second signal peptide domain" which are referred to throughout the claims.
3. Claims 11, 24, 42, and 54 are particularly unclear since they are formulated as dependent claims, although from their content they are independent claims.

PATENT COOPERATION TREATY

PCT

COMMUNICATION OF
INTERNATIONAL APPLICATIONS

(PCT Article 20)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark
Office
(Box PCT)
Washington D.C. 20231
United States of America

Date of mailing:

05 October 1995 (05.10.95)

in its capacity as designated Office

The International Bureau transmits herewith copies of the international applications having the following international application numbers and international publication numbers:

International application no.:

PCT/US95/02576

International publication no.:

WO95/23859

**CORRECTED VERSION
VERSION CORRIGEE**The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra
Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference BWI-120CPPC	FOR FURTHER ACTION <small>see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</small>	
International application No. PCT/US 95/02576	International filing date (day/month/year) 02/03/95	(Earliest) Priority Date (day/month/year) 02/03/94
Applicant BRIGHAM AND WOMEN'S HOSPITAL et al.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ Certain claims were found unsearchable (see Box I).

2. ☐ Unity of invention is lacking (see Box II).

3. ☒ The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing

☒ filed with the international application.

☐ furnished by the applicant separately from the international application,

☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

☐ Transcribed by this Authority

4. With regard to the title, ☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

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☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is:

Figure No. _____ ☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/02576

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C07K16/28 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THE JOURNAL OF EXPERIMENTAL MEDICINE, vol.178, December 1993 pages 2185 - 2192 G.J. FREEMAN ET AL 'Murine B7-2 , an alternative CTLA4 counter-receptor that costimulates T cell proliferation and Interleukin 2 production' cited in the application see the whole document --- -/--	1-3, 18, 33-37, 48-50, 54-56

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
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- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

23 August 1995

Date of mailing of the international search report

01.09.95

Name and mailing address of the ISA

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Fax (+31-70) 340-3016

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Le Cornec, N

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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P,X	JOURNAL OF IMMUNOLOGY, vol.153, no.11, December 1994, BALTIMORE US pages 5038 - 5048 F. BORRIELLO ET AL 'Characterization of the murine B7-1 genomic locus reveals an additional exon encoding an alternative cytoplasmic domain and a chromosomal location of chromosome 16, band B5' see the whole document ---	1-5, 7-13, 15-20, 22-26, 29-31
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A	THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol.100, no.3, March 1993 pages 275 - 281 M. AUGUSTIN ET AL 'Phorbol-12-myristate-13-acetate-treated human keratinocytes express B7-like molecules that serve a costimulatory role in T-cell activation' see page 279, right column	

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference BWI-120CPPC	FOR FURTHER ACTION <small>see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</small>	
International application No. PCT/US 95/ 02576	International filing date (day/month/year) 02/03/95	(Earliest) Priority Date (day/month/year) 02/03/94
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Date of mailing of the international search report

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Name and mailing address of the ISA

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Fax (+ 31-70) 340-3016

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Le Cornec, N

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